Section 2: Vector Biology and Ecology

PLANT AND PREDATOR EFFECTS ON INTERPLANT MOVEMENT BY THE GLASSY-WINGED SHARPSHOOTER

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Reporting period: The results reported here are from work conducted from May 2004 through September 2004.

ABSTRACT
Adult GWSS in caged habitats were monitored hourly to determine the effects of plant species availability and predator presence on intra- and inter-plant movement, as these factors are directly related to the acquisition and spread of Pierce's Disease. GWSS were placed in caged habitats with either a monoculture of beans or polyculture of bean, sunflower, and tree tobacco, and either with or without spiders, in a 2x2 factorial design. Origin of the GWSS (field-caught or laboratory-reared) was also included as a third factor in the multi-factor MANOVA to determine the importance of each treatment on GWSS feeding, resting, and intra- and inter-plant movement. Approximately 85-90% of the day was spent feeding or resting on plants. Only 0.5-1.5% of the observations recorded flying GWSS, and another 1-2% found GWSS walking between plants. More insects moved between plants in the mixed-plant cages than in the bean-only cages, suggesting the GWSS are able to detect the presence of other species of plants in the vicinity. This increase in interplant movement would probably correspond to an increase in Pierce's disease transmission. Field-collected insects spent less time feeding and more time resting on plants than did laboratory-reared insects. Both sets of insects spent more time feeding in bean-only cages than in mixed-plant cages. Beans may not have provided optimal nutrients, and GWSS may have moved to other plants to supplement nutrient intake. GWSS fed on sunflower and tobacco readily, although preferences have not yet been calculated. No predator-mediated spread of Pierce's Disease is expected to occur, as the presence, activity levels, and predation by spiders had no affect on GWSS behavior. Further analysis of feeding times and movement between plant species may clarify the relative importance of toxin dilution (nicotine from tree tobacco) and nutrient balancing from bean and sunflower plants.

INTRODUCTION
The glassy-winged sharpshooter (GWSS) *Homalodisca coagulata* Say, is primarily of economic importance because it vectors the Pierce's disease-causing bacterium, *Xylella fastidiosa* (Blua et al. 1999). The insect feeds on hundreds of species of plants (Adlerz 1980; Hoddle et al. 2003), many of which harbor asymptomatic populations of *X. fastidiosa* (Purcell and Hopkins 1996). Every time a GWSS moves to a new plant to feed, the chances of acquiring and transmitting Pierce's Disease increase. Therefore, the factors causing GWSS to move between plants are directly related to the spread of Pierce's disease.

Generalist herbivores such as the GWSS may move to new plants to balance nutrients, to avoid intra- or inter-specific competition, to dilute plant defensive toxins, or to avoid predation. GWSS feeds primarily, if not exclusively, on the xylem, where nutrients are very dilute (Andersen et al. 2003). The nutritional requirements of GWSS have been determined (Andersen et al. 1992; Brodbeck et al. 1996), and only cowpea and soybean have been found to reliably sustain GWSS throughout a complete generation (D.J.W. Morgan, pers. comm.; Brodbeck et al. 1999). However, why GWSS move between plants, especially when a nutritionally adequate host such as bean is available, is unknown. Interspecific competition is rarely a concern for GWSS, as few other organisms feed on the xylem on the host plants on which GWSS can feed. Intraspecific competition may occur, as GWSS move off plants when present in very high densities (Armer, pers. obs.), but these densities will not occur frequently when biological control is in place. Plant defensive compounds are not common in the xylem (Raven 1983), but alkaloids and quinones are present in certain plant families and may be more prevalent than scientists have previously expected. For example, solanaceous plants carry defensive compounds from synthesis sites in the roots to the leaves via the xylem. Tree tobacco is one such solanaceous plant, which contains nicotine in the xylem. Finally, predators may affect herbivore behavior, as some herbivores can detect and respond to the presence of predators by halting feeding or altering host plant selection (Schmitz et al. 1997; Schmitz and Suttle, 2001). Alternately, an herbivore that moves frequently between plants to optimize feeding may be more apparent to visual predators.

OBJECTIVE
Determine the effect of plant species variety and predators on GWSS interplant movement.

RESULTS
Caged habitats of 0.56m^2 contained 6 plants in soil. Plants and predators were set up in a 2x2 factorial design, with either a monoculture (all bean plants) or polyculture (2 bean, 2 sunflower, and 2 tree tobacco plants) and with or without spiders. Sixteen adult GWSS were placed in each cage and their location and behavior were monitored every hour throughout as daylight was available, for 10-14 hours. The behaviors are shown on the x-axis of Figure 1. The percent of adult GWSS in a cage performing each activity was averaged over all hours observed. The data were compared by a 3-factor MANOVA (SAS

v.8) for differences due to the plant availability (beans-only or mixed plants), spiders (presence or absence), and whether the GWSS were field-collected as adults or lab-reared. Adults that had been reared from birth only on bean plants in laboratory colonies were used in 27 cages, and GWSS that had been captured in the wild as adults were used in 9 cages. One behavior was omitted from the analysis to allow independence of the observations (see Cisneros and Rosenheim 1998).

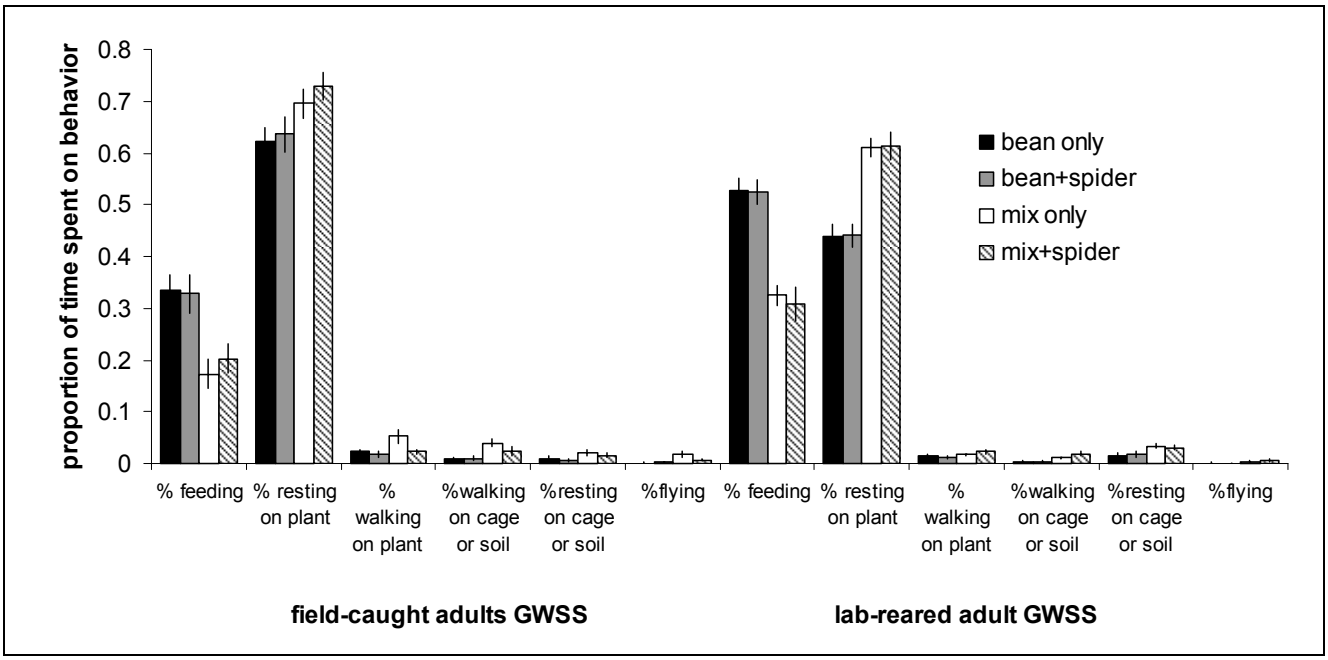

Figure 1. Behaviors performed by caged GWSS adults observed during daylight hours. The average time spent by individuals in each cage on each behavior is shown; error bars indicate standard error.

GWSS spent nearly all of their time either feeding or resting on plants (Figure 1). About 2-5% of the time was devoted to walking on a plant, 1-5% to walking on the cage or soil, 2-5% to resting on the cage or soil, and 0-2% to flying. Plant treatment (bean-only or mixed species) affected all behaviors ($F=13.87$, $df=5,132$, $P<0.0001$). Individuals on beans spent more time feeding and less time resting than insects did on plants in mixed-species cages. Field-caught insects varied significantly from laboratory-reared individuals in their behaviors ($F=16.20$, $df=5, 132$, $P<0.0001$), feeding less and resting more than laboratory insects. However, both groups of insects showed similar time budgets. Both spent less time feeding on beans than on mixed plants. However, lab-reared insects spent less time resting than feeding on beans, and field-reared insects rested more than feeding on beans. This interaction between plant treatment and insect origin (field-caught vs. lab-reared) was significant ($F=2.58$, $df=5,132$, $P=0.029$). Both plant treatment and insect origin significantly affected all insect behaviors at the $p=0.01$ level or greater.

Interplant movement, either by walking or by flying, was higher in the mixed-species cages. GWSS also spent more time resting on the cage or on the soil in the mixed-plant treatment cages, although such a small amount of time was spent in this behavior that it was probably not biologically significant. However, the increase in movement between plants in the mixed cages, although small, is significant in that such behavior increases the GWSS' opportunities to acquire and transmit Pierce's disease.

The three plant species were selected because one provided a host on which GWSS can complete multiple generations (bean), one was an alternate host favored in the field (sunflower), and the final plant contains potentially toxic nicotine in the xylem (tree tobacco), and so may be preferentially avoided. All three plant species were used as host for feeding, but the amount of time spent feeding on each species has not yet been calculated. Both the time spent feeding, and the frequency of leaving each species of plant, will indicate the GWSS' preference for the 3 species.

The presence of spiders did not affect GWSS behaviors ($F=1.08$, $df=5, 132$, $P=0.376$). There were no interactions between spiders and plant species or origin of GWSS. Spiders used in the experiments were field-collected, and the species changed as the season progressed. Predation activity also varied within species, perhaps due to hunger levels of each individual. The presence of spiders did not affect GWSS, but wide variation in spider activity level might hide predation effects. We therefore examined spider activity levels (% of observations in which the spider moved), based on intra- and inter-plant movements, to correlate predation pressure to GWSS movement and feeding behavior. GWSS did not show a behavioral response to spider activity levels (spider activity not correlated to GWSS time spent feeding, moving on the same plant, resting on the plant, moving on the soil or cage, flying) in either plant treatment, nor was the number of GWSS eaten related to spider activity (all non-significant in direct regressions). The spiders were equally active in the two plant treatments,

moving an estimated 28±3% (mean±SE) of the observation period in both treatments. Spiders in the bean treatment caught and fed on 0.22±.07 GWSS per day, whereas those in the mixed-plant treatment fed on 0.33±.09 GWSS.
All GWSS were sexed after observation, and data were examined for possible behavioral differences. However, there were no differences between the sexes in terms of their behavior (MANOVA with sex and plant-spider treatment as the factors; $F=1.29$, $df=5,276$, $p=0.27$).

CONCLUSIONS

The availability of multiple plant species increased GWSS interplant movement, and feeding times were reduced in these cages, suggesting GWSS 1) can detect the presence of other host species in the vicinity, probably through olfaction, and 2) that diet-mixing helps GWSS obtain needed nutrients more rapidly. However, the increased movement between plants also may correspond to an increased in acquisition and spread of the bacterium that causes Pierce's Disease. The effects of potentially toxic plants, such as tree tobacco, are not currently understood on GWSS interplant movement. Further data analysis should help clarify the insects' response. Spiders did not affect GWSS feeding and intra- and inter-plant behavior in the observations described here. Thus, these (and possibly other arthropod) predators should not affect the GWSS' acquisition and spread of Pierce's Disease.

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SHARPSHOOTER FEEDING BEHAVIOR IN RELATION TO TRANSMISSION OF THE PIERCE'S DISEASE BACTERIUM

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ABSTRACT
Progress this year consisted of completing past projects as well as building infrastructure for future research. Backus's new lab in Parlier was renovated, upgraded and equipped with state-of-the-art facilities for electrical penetration graph (EPG) monitoring of insect feeding and histology of plant and insect tissues. Extensive colonies of glassy-winged, smoke tree, green, and red-headed sharpshooters were established in Fresno and Parlier (with R. Groves, ARS Parlier). New personnel were hired; data was intensively analyzed and grant proposals written. Much effort was also expended in developing new protocols and preliminary findings for feeding waveform correlations with bacterial expulsion and muscle contraction, as well as AC and DC waveforms for several species in colony. Stylet activities and salivary sheath-cell type correlations for the major GWSS waveforms were completed (Objective 1), as was all of the plant histology for the GWSS inoculation test (Objective 2). Results to date support a modified version of last year's hypothesis for the mechanism of *Xf* inoculation to grape. *Xf* bacteria may exit the stylets during brief stylet activities represented by the B1spikelet burst, B1-like portions of N and/or C, probably within seconds of the first puncture of any penetrated cell, both along the pathway to and within xylem. Proper placement of the bacteria appears to be crucial; placement in xylem leads to growth of the bacteria sufficient for detection by less sensitive methods such as culturing. Otherwise, when more sensitive detection methods such as immunocytochemistry of the tissues immediately surrounding the salivary sheath are used, they can detect *Xf* in non-xylem tissues. Three papers from this research are in preparation for submission in late 2004 – early 2005. This work will help solve the PD/GWSS problem by identifying the mechanism of *Xf* inoculation and crucial aspects of inoculation efficiency, and eventually aid host plant resistance through the development of the Stylet Penetration Index.

INTRODUCTION
Almost nothing was known, until this work, about the stylet penetration behaviors of the glassy-winged sharpshooter (GWSS), and how they interact with populations of *Xylella fastidiosa* (*Xf*) to facilitate transmission to grapevine. This project is combining the three most successful methods of studying leafhopper feeding (i.e. histology of fed-upon plant tissues, videotaping of feeding on transparent diets, and electrical penetration graph [EPG] monitoring) to identify most details of feeding.

OBJECTIVES
1. Identify and quantify all feeding behaviors of GWSS on grapevine, and correlate them with location of mouthparts (stylets) in the plant and presence/ population size of *Xf* in the foregut.
2. Identify the role of specific stylet activities in *Xf* transmission, including both the mechanisms of acquisition and inoculation, and their efficiency. This project's emphasis is on inoculation.
3. Begin to develop a simple, rapid method to assess feeding, or detect the likelihood of *X. fastidiosa* transmission (an "inoculation-behavior detection method"), for future studies.

RESULTS
During the first six months of this reporting period (Nov. 2003 – April 2004), Backus's new lab at USDA-ARS in Parlier was closed due to extensive renovation construction underway. Notwithstanding this delay, we made significant progress on several sharpshooter research fronts during this time. We hired new personnel (a post-doc and a second technician), purchased many supplies and pieces of equipment (including a new confocal microscope), and trained in the use of the equipment. Also, we received CDFA importation permits and permission for a GWSS maintenance colony to be established in Fresno Co., at a site on the campus of CSU-Fresno. A trailer was rented, retrofitted for quarantine infrastructure, and inspected by officers of the Fresno Co. Agricultural Commissioner's office. Insect maintenance and research rooms were built and outfitted with lighted shelves, cages, growth chambers, and research equipment. Also, a contract was arranged by Groves and Civerolo with Morgan to supply greenhouse-reared GWSS on a monthly basis. Acquisition of insects began in

September 2004. The new USDA-ARS/CSU-Fresno Insect Maintenance and Research facility went into full operation in October 2004. Also during this time we established colonies in the greenhouse in Parlier of the following species: smoke tree sharpshooter, *H. liturata* (STSS), as well as (with Groves) red-headed sharpshooter, *Xyphon fulgida* (RHSS), green sharpshooter, *Draeculacephala minerva* (GSS) and three-cornered alfalfa hopper, *Spissistilus festinus* (3CAH) (collected locally). Preliminary studies of the feeding behavior and EPG waveforms of all of these species are underway.

In addition to major infrastructure improvements in the first 6 months, we also analyzed past data, and Joost performed extensive preliminary tests to develop new protocols in electromyography and real-time imaging of sharpshooter muscles controlling feeding. We also wrote papers, and reviewed and wrote grant proposals. Among these were revisions of the Almeida & Backus paper on blue-green sharpshooter waveforms, now in print [1] and a newly funded UC PD proposal to continue research on mechanisms of *Xf* transmission and details of ingestion behavior. Once we had moved back into the lab and set up, progress resumed on existing objectives during the last four months of the reporting period (July – October 2004).

Objective 1 - Waveform Correlations

Experiment 1: AC-DC Correlation Monitor

Significant progress was made this year in the continuing development of this technology. Bennett built two new prototype monitors, the last of which included design suggestions developed by Backus in consultation with W. F. Tjallingii, Wageningen Agricultural University, The Netherlands. These prototypes for the first time succeeded in achieving waveform fidelity with the original, separate AC and DC waveforms, a goal sought for the last two years of work developing these instruments [2].

Experiment 2: Salivary Sheath-Cell Type Correlation

Backus analyzed histological images produced last year by Habibi from recordings made by Yan (see methods and preliminary findings in [2, 3]). Preliminary findings and waveform appearances are the same as those pictured in the 2002 and 2003 progress reports [2, 3], but waveform names are as in [3]. Results show that early pathway activities, especially A1, occur in the shallow epidermal/parenchyma tissues, A2 and continuous B1 usually occur in the parenchyma peripheral to the vascular bundle (although the sample size of tissues collected for B1 is very small). B2 usually occurs in the parenchyma or phloem, and is often associated with a large deposit of sheath saliva sometimes at a branching point in the sheath. The number of B2 events is also correlated with the number of sheath branches. Short, early C and N events can occur variably, in parenchyma, phloem or xylem; however, longer later C and N events are almost always in mature xylem cells. It is still uncertain whether B1 or C may represent the first penetration of a xylem cell. Correlations were completed and a manuscript is *in prep* for submission in late November [4]. Appendix Table A further summarizes the plant tissue/cell correlations known at the end of the reporting period (late Sept. 2003).

Experiment 3: Stylet Activities Correlation

Joost analyzed the videomicrography data collected by Yan of the stylet activities in artificial diet (see methods and preliminary findings in [2, 3], as well as a schematic of the equipment in the Backus et al. 2004 poster). Stylets could clearly be seen performing stereotypical behaviors during three waveform types frequently seen on grape, i.e. A1, A2 and B1. Results are summarized in Figures 1 – 4 below, Table A and in the Backus et al. 2004 poster. They reveal for the first time that A1 represents the primary formation of the salivary sheath (Figures 1, 2), B1 represents stylet tip fluttering (Figures 1, 3), and B2 represents stylet sawing through the hardened sheath (and, we speculate, perhaps also through tough plant material) (Figures 1, 4). It is particularly interesting that the B1 spikelet burst is dispersed intermittently throughout other pathway waveforms, e.g. between peaks of A1 (Figure 2), as well as in continuous durations by itself (Figure 3). This dispersion, plus last year's Experiment 4 finding [3] that B1 was the only pathway waveform associated with *Xf* inoculation, suggest that the spikelet bursts might represent precibarial valve movement, an important component of a hypothesized inoculation behavior [4]. A manuscript describing these results is *in prep* for submission in late November [5].

Objective 2 - Inoculation Behavior:

Experiment 4: EPG Waveforms Associated with Inoculation

Habibi completed sectioning and photomicrography of the remaining grape tissues probed by EPG-recorded GWSS, i.e. those during the short probe treatment (see the 2003 progress report [3] for methods and preliminary findings). Results from each of the three bacterial detection methods used (Table 1) continue to support that immunocytochemistry may be the most sensitive detection method; 56% of probes showed positive detection of *Xf* near the salivary sheath, while 45% were positive with PCR, and only 10% with culturing. These findings continue to support the interpretations discussed in the 2003 progress report [3]. Unlike PCR, immunocytochemistry results suggest that detectable bacteria are inoculated more often during long than short probes (Table 1). However, it will be important to determine how many insects were actually inoculative before we can state that conclusively. We have begun to dissect the fixed, dried heads of the recorded sharpshooters for scanning electron microscopy, to determine how many of them contained *Xf* and in exactly which areas in the precibarium/cibarium. This information will be

Table 1: Number of EPG-GWSS-probed grape samples that was positive for *Xf* near the probe out of the total number tested, for each of the three bacterial detection methods.

Probing Treatment	PCR	Culture	Immunocyt.
3 short probes	5/10	0/10	3/8
1 long probe	4/10	1/8	6/8

correlated with all other findings to determine how often the inoculation behavior, when performed by bacteria-laden insects, actually results in expulsion of *Xf*. Present findings [3] still implicate waveforms B1, C and N, especially during long probes. All data analysis will be completed and a manuscript submitted in early 2005 [6].

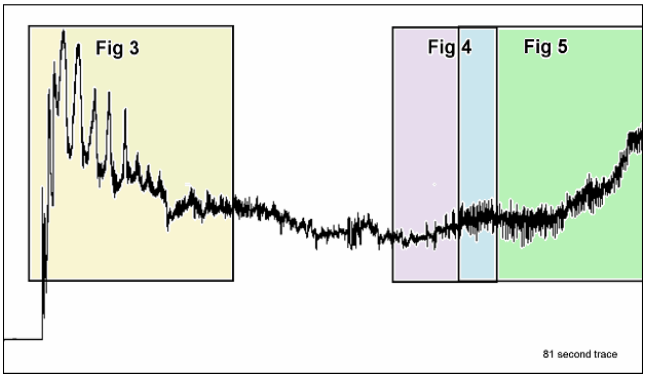

Figure 1. Waveform of GWSS probe in artificial diet compressed 35 times. Box labels indicate where Figures. 3-5 were taken from this trace.

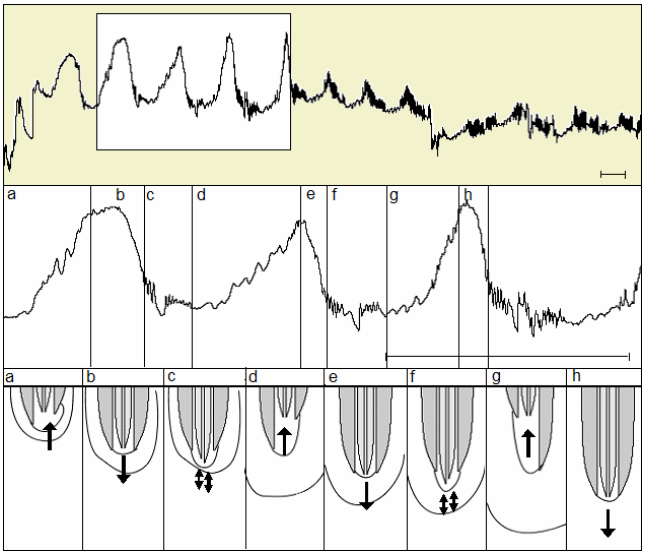

Figure 2. A1 waveforms were correlated with GWSS stylet activities in artificial diet. Top panel trace contains an A1 waveform compressed 5 times. The middle panel is an uncompressed A1 waveform trace that corresponds to the boxed waveform trace in the top panel. Subdivisions, a-h, in middle panel are correlated with stylet activities in the bottom panel with the same subdivision letters. Time marks in the lower right hand corner of the top and middle panel equal one second.

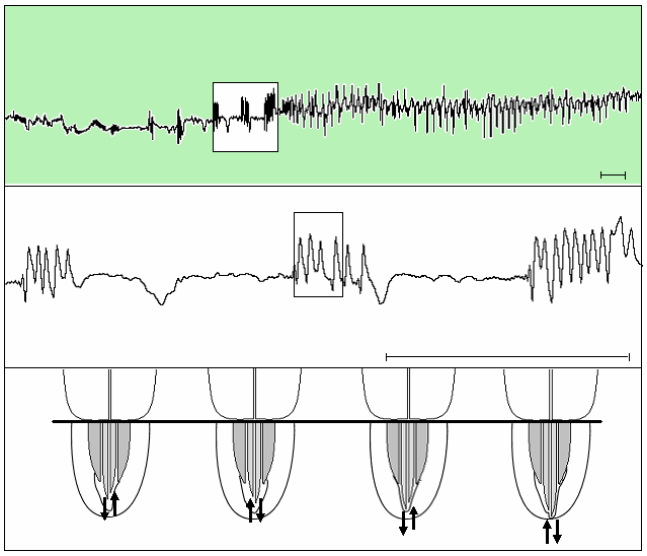

Figure 3. Correlation of B1 waveforms with GWSS stylet activities in artificial diet. Top panel is a waveform trace with B1 compressed 5 times. The middle panel is an uncompressed B1 waveform trace that corresponds to the boxed waveform portion in the top panel. The boxed waveform portion of the middle panel is a B1 spikelet burst and correlates with the stylet activities in the bottom panel. Time marks in the lower right hand corner of the top and middle panel equal one second.

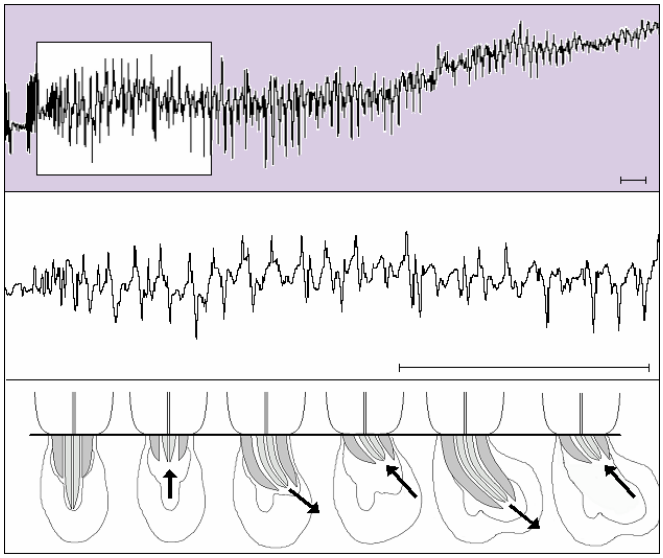

Figure 4. Correlation of B2 waveform with GWSS stylet activities in artificial diet. Top panel is a B2 waveform trace compressed 5 times. The middle panel is an uncompressed B2 waveform trace that corresponds to the boxed portion of the waveform in the top panel. The bottom panel are the stylet activities that were observed at the onset of the B2 waveform and through out the waveform. Time marks in the lower right hand corner of the top and middle panel equal one second.

CONCLUSIONS

These findings will help solve the PD/GWSS problem by:

- Identifying the mechanism of *Xf* inoculation and using EPG to observe it real-time as it occurs,
- Identifying one determinant of inoculation efficiency, i.e. the role(s) of inoculation behavior vs. bacterial presence and/or detachment in the foregut,
- Developing protocols for further tests of transmission biology and efficiency, especially with respect to acquisition.
- Developing a Stylet Penetration Index for testing among host and non-host species or cultivars, diets, etc. for performance of transmission behaviors, ultimately leading to improved host plant resistance.

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Appendix Table A. Current definitions of the AC EPG waveform phases, families and types of GWSS on grape.

Waveform Phase	Waveform Family	Waveform Type	Waveform Characteristics	Proposed Biological Meanings	
				Plant Tissue/Cell	Insect Activity
Pathway	A	A1	Highest amplitude, hump-like waveform at beginning of probe; usually with spike at the top	Parenchyma or mesophyll	Major salivary sheath formation; deep extension/ retraction of stylets; some watery salivation
		A2	Medium amplitude, variable slope; irregular, high frequency with occasional trenches and/or potential drops	Parenchyma or mesophyll	Lengthening and/or hardening of salivary sheath; cell membrane breakage; some watery salivation
	B	B1	Short, single- or multi-peak "spikelet bursts" (20-28 Hz) separated by flatter, wave-like sections	Parenchyma or xylem or pith	Stylet tip fluttering; possible internal muscle/valve movement; involved in inoculation
		B2	Extremely regular, stereotypical pattern of peaks (6 Hz), with distinct phrases	Parenchyma or xylem or pith	Stylet sawing through salivary sheath or tough wood; sheath branching; sheath salivation
Ingestion	C	C (to be subdivided)	Very regular, low rep. rate (3 Hz) with with distinct phrases	Parenchyma or xylem or pith	Trial (short) or sustained (long) ingestion (watery excretory droplets correlated)
Interruption	N	N (to be subdivided)	Irregular, appearing A-like at times, but interrupting continuous C; ave. dur. 16 sec.	Parenchyma or xylem or pith	Sheath or watery salivation in ingestion cell; sheath extension

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EFFECTS OF FEEDING SUBSTRATE ON RETENTION AND TRANSMISSION OF *XYLELLA FASTIDIOSA* STRAINS BY THE GLASSY-WINGED SHARPSHOOTER

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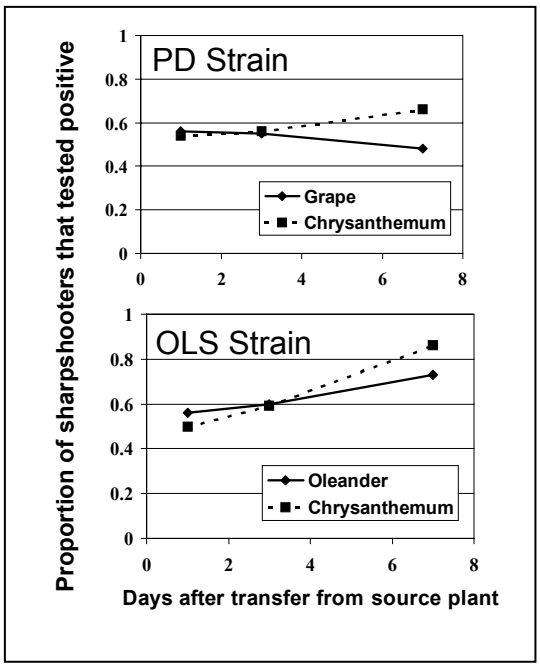
Reporting Period: The results reported here are from work conducted from October 2003 to September 2004**.**

ABSTRACT
In this project we are testing the effects of feeding substrate on the acquisition and retention of *Xylella fastidiosa* by the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*. We are using two strains of *X. fastidiosa* that are present in California: a Pierce's disease (PD) strain that infects grape, and an oleander leaf scorch (OLS) strain that infects oleander. A series of experiments were conducted to compare the retention of PD or OLS strains after acquisition, when insects were subsequently maintained on a plant species that was either a host or non-host of that particular strain. In these studies, we found no significant difference in the mean proportion of insects testing positive for the PD or OLS strains, regardless of whether the insects were subsequently fed on either a host or a non-host of the PD or OLS strain. Thus, retention of a particular strain of the pathogen by an individual insect does not appear to be dependant on the xylem content of the plant host on which it is feeding. In a second study transmission efficiency of adult GWSS fed for 24 h on *X. fastidiosa*-infected plants was compared to those fed for 24 h on *X. fastidiosa* from pure media-grown cultures delivered through a cut stem system. In these experiments insects transmitted PD and OLS strains when they acquired the bacteria from a plant, but did not transmit either strain when media-grown bacteria were delivered through the cut-stem system.

INTRODUCTION
The glassy-winged sharpshooter (GWSS) is capable of acquiring and transmitting several different strains of *X. fastidiosa* from a variety of host plants. In this project we are testing the effects of feeding substrate on the acquisition, retention and transmission of *X. fastidiosa* by GWSS. Two strains of the pathogen present in California are being used in these experiments: a Pierce's disease (PD) strain that infects grapevine, and an oleander leaf scorch (OLS) strain that infects oleander. These two strains have different host ranges; the PD strain does not infect oleander, and the OLS strain does not infect grape.

OBJECTIVES
1. Compare retention times of *X. fastidiosa* when infected glassy-winged sharpshooter (GWSS) are subsequently fed on plants that are either hosts or non-hosts of the strain they carry.
2. Compare acquisition and transmission efficiency of insects fed on infected plants to those fed on media-grown cultures delivered through cut stems.
3. Compare retention times of two strains of *X. fastidiosa* in GWSS when simultaneously acquired through cut stems, then subsequently fed on either (a) a non-host of both strains, (b) on a host of only one strain, or (c) alternating hosts of each strain.
4. Test the effects of antibacterial materials on acquisition and transmission of *X. fastidiosa* by GWSS.
5. Test the effects of variation in substrate pH and free ion availability on the acquisition and transmission of *X. fastidiosa* by GWSS.

RESULTS
Objective 1
We began by comparing the relative proportion of insects that tested positive after acquisition of a given strain of *X. fastidiosa,* when they were subsequently maintained on a plant species that was either a host or non-host of that strain. Grape plants (*Vitis* spp.) infected with a Pierce's disease (PD) strain of *Xylella fastidiosa*, and oleander plants *(Nerium oleander*) infected with an oleander leaf scorch (OLS) strain were used as sources of inoculum. The strain of *X. fastidiosa* infecting plants was confirmed by PCR. Groups of GWSS adults were caged on either an OLS infected oleander plant, or a PD infected grapevine for 2 days. Insects were then moved to an uninfected plant of the same species as the source plant (oleander or grape), or to a non-host of the strain (chrysanthemum). Samples of insects were collected at 1, 3, and 7 days after transfer to uninfected hosts and frozen. Insects were subsequently tested for the presence of *X. fastidiosa* using PCR.



Results from retention experiments using the OLS strain acquired from oleander showed no significant difference in the mean proportion of insects testing positive when insects were subsequently fed for on either a host (oleander), or a non-host (chrysanthemum) of the OLS strain for 1, 3, or 7 days after acquisition. Similarly experiments using the PD strain acquired from grapevine also found no significant difference in the mean proportion of insects testing positive at 1, 3 or 7 d after acquisition regardless of whether the insects were subsequently fed on either a host (grapevine) or a non-host (chrysanthemum) of the PD strain. Thus, both PD and OLS strains of *X. fastidiosa* remained detectable in GWSS, even when the insects fed on a non-host of the strain for 7 d.

Objectives 2 and 3

To test if feeding substrate can influence the ability of insects to acquire and transmit a particular strain of *X. fastidiosa*, we plan to use a pathogen delivery system to allow us to either maintain or manipulate the feeding substrate as desired. The method described by Bextine and Miller (2002) was originally used for an *Alcaligenes* sp. of bacteria. This technique was modified to provide an environment suitable to survival of *Xylella fastidiosa* and the test plants used. We are using sections of chrysanthemum stem about 10 cm long that are connected by tubing to a syringe with a suspension of *X. fastidiosa* in PBS. The distal end of the stem is also cut and left open. The syringe is depressed until liquid is extruded from the distal end of the cut stem. Then GWSS are allowed to feed on these stems.

To demonstrate that live *X. fastidiosa* cells could survive movement through a cut stem, *X. fastidiosa* was suspended in a PBS buffer, and the syringe was depressed until liquid was extruded from the distal end of the cut stem. Droplets forming on the distal end were collected and analyzed using PCR to determine if *X. fastidiosa* cells were present. In all cases, *Xylella* was detected within the first 10 drops extruded. Thus, in these experiments, material was injected into stems until at least 10 drops of material was extruded from the distal cut end to ensure that the bacteria have been moved the entire distance of the stem.

In transmission experiments, adult insects were fed for 24 hours on either infected plants, or media-grown bacteria delivered through the cut stem system as described above. Adults were then individually moved to uninfected test plants and allowed to feed for 4 d. When GWSS adults were fed on PD-infected grapevines, 12/26 (46%) transmitted the pathogen to healthy grapevine test plants. In contrast, when insects were fed on media-grown PD bacteria through the cut stem method, no individuals (0/48) transmitted the pathogen to test pants. Similar results were found with OLS-infected plants (9/37,or 24% of individuals transmitted) compared to media-grown OLS delivered through cut stems (0/22 transmitted). Thus, insects did not transmit PD or OLS strains when media-grown bacteria were delivered through the cut-stem system. Purcell et al. (personal communication) found similar results when leafhoppers were fed *X. fastidiosa* through parafilm sachets.

Additional studies are being conducted to determine why insects are unable to transmit the pathogen from the cut stem delivery system. For example, a recent study demonstrated that *X. fastidiosa* cultures will produce different levels of "biofilm formation" when grown on different types of media (Leite et al. 2004). We will test if growing our strains on different media may help induce transmissibility by insects. In addition, we will conduct further studies to determine the pathway of the bacteria through the system. For example, by testing the honeydew of insects feeding on the cut stem system, we can determine if the bacteria are successfully passing through the insect. In the interim, work on the remaining objectives will continue using insects fed on PD and OLS infected plants.

CONCLUSIONS

In retention experiments (Objective 1) for both the PD and OLS strains, we found the proportion of insects retaining the pathogen was the same, regardless of whether insects subsequently fed on a host or a non-host of that strain. This indicates that retention of a particular strain of the pathogen by an individual insect is not dependant on host-specific xylem content of the plant on which it is feeding. In transmission experiments (Objectives 2 and 3) insects successfully transmitted the PD and OLS when they acquired the pathogen from infected grapevine and oleander plants respectively, but did not transmit either the PD or OLS strains when the media-grown bacteria were delivered through the cut-stem system. This could be the result of biological characteristics of media-grown bacteria that contribute to non-transmissibility by insects, or failure of the cut stem system to properly deliver bacteria to the insect. Further experiments are being conducted to determine the basis for lack of transmission of media-grown bacteria by GWSS.

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FUNDING AGENCIES

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DEVELOPMENT OF AN ARTIFICIAL DIET FOR THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT
The intent of this project is to develop an artificial rearing system for the glassy-winged sharpshooter (*Homalodisca coagulata*) (GWSS), the primary vector of Pierce's Disease (*Xylella fastidiosa*) (PD). In order to accomplish this, a diet delivery system will first be developed and then used to test artificial diets. Diet formulations will be based, in part, on previous studies performed by Cohen (2002) using GWSS, as well as on artificial diets developed for other Hemiptera (Mitsuhashi, 1979; Coudron *et al*., 2002) and on the xylem chemistry of GWSS host plants (Andersen, et al., 1992). Diets will be evaluated based on their effects on life history analyses, reproductive rate and intrinsic rate of increase of GWSS. Another aspect of our project involves investigating nitrogen source(s) for GWSS, as that may represent a nutrient limitation for xylem feeders. Two potential sources for nitrogen, i.e. proteins or peptides, will be studied by determining the fate of dietary proteins/peptides (Brandt, et al., 2004) and the ability of salivary and midgut proteolytic enzymes to digest proteins/peptides (Wright, et al., 2004). In this way, we will identify the role(s) proteins and peptides play in GWSS nutrition and their potential uses in artificial diet formulations.

INTRODUCTION
The formulation of an artificial diet for GWSS will greatly enhance the ability of researchers to rear this insect. Presently, the rearing of GWSS is labor-intensive and costly because of its dependence on the propagation of appropriate host plants, with researchers often needing to propagate several species of plants to enable them to rear GWSS under optimal conditions. The development of an artificial diet would likely be more cost effective and portable, increasing the availability of high quality insects for Pierce's disease researchers and decreasing the costs and time-constraints associated with maintaining the insect in culture. The increased accessibility of GWSS to researchers can lead to more rapid developments in novel control measures for this major vector of PD, with these new measures being directly applied by growers. Furthermore, the coupling of an artificial diet with a suitable delivery system can lead to an improved understanding of the relationship between GWSS nutrition and other PD-related issues (including GWSS' varying abilities to acquire/maintain/transmit infectious *Xf* under different circumstances, e.g., via artificial membranes vs. plants, Redak et al., 2004). In addition, the diet delivery system alone would have other potential uses such as in studying the interactions between GWSS, *Xf*, and the host plant, as well as in testing potential anti-GWSS and anti-*Xf* control agents. This could be accomplished by incorporating into the feeding system: 1) selected host plant-associated compounds; 2) media containing the causative agent of PD (*Xylella fastidiosa*, *Xf*) (although some studies have suggested that *Xf* acquired via an artificial membrane by GWSS may not be infectious, Redak et al., 2004); 3) control agents including anti-GWSS or -*Xf* compounds (such as proteins to be engineered into host plants to control either GWSS or *Xf*; Dandekar et al., 2003; Lin, 2003; Meredith and Dandekar, 2003; Reisch et al., 2003) or anti-GWSS microbials (Kaya, 2003; Mizell & Boucias, 2003). In summary, the development of an artificial diet and a corresponding delivery system for GWSS could lead to insights that can be used to generate improved methods for controlling GWSS and, therefore, Pierce's disease.

An important part of our project also involves gaining a better understanding of the digestive physiology of GWSS. This will be investigated by focusing on the role proteins and peptides play in GWSS nutrition, as these or similar compounds have been isolated from some xylem fluids (Cohen, 2002; Jain and Basha, 2003; Rep et al., 2003). We will accomplish this by determining the extent to which GWSS can digest proteins and peptides, as well as elucidating the fate of specific ingested proteins in GWSS. This information will be directly used in the generation of an optimal artificial diet for GWSS. Furthermore, GWSS' ability to degrade proteins/peptides will also shed light on the degree to which GWSS can disable defensive proteins/peptides in plants, which is important when dealing with salivary enzymes that are secreted into plant tissues and could alter anti-*Xf* defense components (e.g., either naturally occurring or genetically engineered proteins/peptides; Lin, 2003; Meredith and Dandekar, 2003; Reisch et al., 2003). This knowledge could be used when modifying target plants such as grapevines to improve their resistance against Pierce's disease (PD). Therefore, our investigation into nutritional requirements will not only aid us in the development of a suitable artificial diet for GWSS, but

will also provide insights into the potential efficacies of anti-PD plant modifications.

OBJECTIVES

1. Develop an artificial diet delivery system for rearing the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata.*
2. Formulate and evaluate an artificial diet for the development and reproduction of GWSS.
3. Investigate the utilization of proteinaceous components in the food stream of GWSS in order to refine and improve the artificial diet using physiological and proteomic/genomic approaches.

RESULTS AND CONCLUSIONS

This project has just been funded. Preparation of quarantine facilities is complete and the identification of insect cultures to be used in our studies is underway. The process to hire an additional researcher has been initiated. Preliminary experiments, in collaboration with Jones and Setamou at ARS in Weslaco, have demonstrated continuous feeding by adult GWSS for over 30 days on artificial diets presented through a specialized feeding tube. Additionally, differences in survival have been noted as a result of changes in amino acid concentration and composition within the diet.

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BIOLOGY AND ECOLOGY OF THE GLASSY-WINGED SHARPSHOOTER IN THE SAN JOAQUIN VALLEY

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ABSTRACT

We followed glassy-winged sharpshooter (GWSS) preference and age structure on ornamental host plants in Bakersfield, California. Results of an urban survey showed GWSS host utilization varied greatly. This was especially true during the growing season when the mobile GWSS nymphs and adults would frequently shift amongst abutted host plants. While host plant utilization was dynamic, yet there were clear seasonal patterns. In late-fall through mid-winter, GWSS were most commonly found on privet, oleander, and citrus. In late-winter through spring, the preferred hosts were Xylosma, photinia, and flowering pear. In summer, host utilization was most dynamic and often dependent on host condition (such as irrigation). Nevertheless, GWSS adult and nymph summer and early-fall populations were consistently found on Xylosma, photinia, oleander, star jasmine, and Crape myrtle. Controlled experiments with potted host plants found similar results and highlight differences in GWSS feeding and oviposition preferences. Throughout all studies, we sampled the numbers of predators and parasitoids. Emerged parasitoids show *Gonatocerus ashmeadi* and *G. triguttatus* were reared from egg masses collected on most host plants, and accounted for a large percentage of summer GWSS mortality. Predators were present, especially spiders, and often observed feeding on GWSS. However, our data has not yet found any one predator species to be consistently associated with GWSS or with a reduction in GWSS densities. Collected predators are being analyzed using immunologically-based assays that employ pest-specific monoclonal antibodies (MAbs) to help identify the key predators of GWSS. During the urban surveys, we collected plant material (e.g., potential vector host plants) and potential insect vectors to determine the incidence of *X. fastidiosa*. This material was processed in the laboratory using "immunocapture DNA extraction" to determine the presence of *X. fastidiosa*. Results show that GWSS collected in urban regions often (>10%) carry *Xylella fastidiosa*, however, it is not the strain that cause PD.

INTRODUCTION

The primary focus of this research is the description of glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, GWSS preference, egg deposition, age structure, population dynamics and levels of natural regulation on different host plants in the urban / agricultural interface in the San Joaquin Valley (SJV). Currently, such a description of GWSS biology and ecology in the SJV is lacking. The developed information from this research will help understand GWSS seasonal movement and infestation foci. Of primary concern to regional control programs is whether or not untreated urban GWSS populations serve as an inoculum source for either the insect vector or the bacterial pathogen, *Xylella fastidiosa* (*Xf*).

To develop a more complete description of host plant influence on GWSS age structure and natural enemy impact, we conducted both urban surveys and manipulative experiments. Specifically, we sought to determine the potential of common plant species used in residential landscaping to either reduce or increase GWSS densities. We further screened common plants and GWSS collected for the presence of *Xylella fastidiosa*. When completed, information on the abundance, host plant use, and seasonal dispersal patterns of GWSS and natural enemies in urban better enable researchers to determine GWSS movement and host plant succession in the SJV, and the data may be useful for modification of surrounding vegetation, such as trap crops, to suppress GWSS movement into a vineyard.

OBJECTIVES

1. Determine glassy-winged sharpshooter biology and ecology throughout the season, particularly its age structure on and utilization of the different host plants that represent common breeding or dispersion refuges for glassy-winged sharpshooter in the San Joaquin Valley.
2. Determine the contribution of resident natural enemies on glassy-winged sharpshooter mortality and whether natural enemy abundance or species composition varies significantly on different GWSS host plants or ecosystems in the San Joaquin Valley.
3. Determine the presence of *Xylella fastidiosa* in glassy-winged sharpshooter collected from different host plant species and in selected ecosystems in the San Joaquin Valley.

RESULTS

Objective 1 - Survey.

GWSS numbers, age structure and natural enemies were surveyed in residential areas in Bakersfield, California. In the 2003-2004 season, six residential sites were sampled. Each site was selected for its combination of different GWSS and *Xf* host plants; most of the sampled sites had 3-8 individual plants of each plant species, with 3 or more GWSS host plant species in close proximity. Host plants surveyed included: carob, rose, star jasmine, Chinese elm, flowering pear, apple, escallonia, pink lady, ivy, nectarine, photinia, citrus, gardenia, privet, euonymous, hibiscus, agapanthus (lily of the Nile), grape, crape myrtle, eucalyptus, mock orange, oleander, Xylosma and Wheeler's dwarf. Each month, samples were taken for GWSS and natural enemies. We also recorded plant condition. From April 2003 to October 2004, we made >3000 plant samples (sample plant × sample date).

A thorough analysis of this data set will be made at the end of the residential survey (April 2005) when we project to have >5000 samples, each with information on host plant species, condition and phenology; GWSS density and age structure; and potential natural enemies present. An initial analysis show strong host plant preferences GWSS adults and nymphs, especially towards oleander, crape myrtle and Xylosma during the spring and summer months (Figure 1). Host plant preference for adult and nymph feeding sites was not always the same as those preferred for egg deposition – especially with respect to oleander, as reported by other researchers.

The seasonal population dynamics showed a strong spring GWSS population on all hosts followed by a summer decline, which is largely attributed to egg parasitism of the summer brood. We believe that the winter period is critical for GWSS population dynamics as this period represents the low point in the population density. Oleander and privet may be the most important overwintering hosts in the urban regions. In contrast, host plants as crape myrtle and crabapple are dormant throughout winter and, according to our samples, play no role in the GWSS overwintering. However, they are excellent hosts for oviposition and nymphal development during late spring and summer time. For some host, GWSS are confined to specific sections. For example, the flowering pear trees brake dormancy early in the year and start blooming by the first week of February. GWSS adults have been found on the twig tips in the middle of the winter in these trees. It is unknown whether they survive the entire winter in this plant or the early physiological activity of the flowering pear attracts the GWSS. We also found GWSS overwintering exclusively on the "suckers" of the following tree species: eucalyptus, carob tree, Chinese elm, and olive.

Objective 1 – Manipulative Experiments

To categorize GWSS age structure, ecology, and resident natural enemies (particularly predators) on different host plants common in urban areas, potted (6.6 L) plants were used to provide a replicated array of similarly-conditioned (e.g., age, size, irrigation) GWSS host plant species. These preference studies were conducted in an unsprayed, GWSS infested citrus orchard, and two unsprayed residential areas in Bakersfield, California. Perennial species included ivy, photinia, citrus, gardenia, privet, euonymous, hibiscus, agapanthus (lily of the Nile), grapevine, crape myrtle, eucalyptus, and oleander. Annual (or weed) species included prickly lettuce, little mallow, annual sowthistle, coast fiddleneck, common groundsel, London rocket, fox tail brome, lambsquarters, blue grass, and shepperd purse. Both perennial and annual species were set in a randomized block design. Results show GWSS seasonal-long densities were influenced by host plant species, with a significant difference (ANOVA, $P < 0.001$) among host plants, for both perennial and annual categories (Daane et al. 2003. 2004a). Results are provided for perennial host plants in the citrus orchard (Figure 2), which shows a 20-fold difference in the number of GWSS on ivy, the least preferred host planted tested, and grape, the most preferred. We found a relatively similar pattern in the 2002/03 and 2003/04 seasons. Interestingly, GWSS egg mass density was not related to adult or nymphal densities ($P = 0.25$, $r^2 = 0.03$; $P = 0.35$, $r^2 = 0.01$, respectively). As with the urban survey, we conclude that GWSS adults have oviposition preferences that may be different from the nymphal feeding preference. We believe this difference is a result of both GWSS adults and nymphs switching among host plants, and to a disparate level of predator and parasitoid activity.

In a second experiment, we manipulated combinations of GWSS host plant species in cages. Four plant species have been planted in different combinations (e.g., citrus only, citrus and oleander, oleander only, oleander, citrus and crape myrtle), with a total of 7 plant species (4 replicates). Initial progress was slowed by the difficulty we encountered in transferring field-

collected GWSS material to the experimental site – basically, many of the GWSS nymphs died or left the tested host plant almost immediately after being transfer. We are currently improving inoculation techniques.

Objective 2 – Natural Enemies

During the surveys of GWSS population dynamics in non-agricultural regions, described previously, we collected information on GWSS natural enemies, using sampling techniques such as GWSS egg mass collections (>100 leaves per perennial plant species per collection) and potential GWSS predator collections (beat and sweep samples). As in all studies, we recorded host plant species and seasonal period. We found *Gonatocerus ashmeadi* and *G. triguttatus* (Triapitsyn et al. 1998) comprised about 95 and 4%, respectively, of collected parasitoids. As has been suggested, these parasitoids kill >90% of the summer GWSS population. Parasitoid numbers drop during the winter, when most GWSS are in the adult stage – although large nymphs were present as well. No egg masses or recently hatched nymphs were found from November through February. The first fresh egg masses were collected in April (2003) and March (2004), and we found parasitized eggs within as soon as April (2004). Our results suggest that egg parasitoids are the primary biological control factor. Combined with the winter / spring area wide insecticide control programs (which dramatically reduce the over-wintered population on citrus, the primary GWSS host plant during this period, and lower the overall GWSS population levels in the SJV) the egg parasitoids reduce the GWSS population in the urban regions to such an extent that GWSS can be difficult to find in large numbers in late summer samples.

Predators may play a small role controlling GWSS nymphs. Spiders were the most common predator found, and there was a significantly positive relationship between the number of spiders found and the number of GWSS egg masses ($P < 0.001$, $r^2 = 0.28$). Still, there has not yet been any concrete evidence that links these generalist predators with the regulation or suppression of GWSS. During the GWSS urban surveys, predators were collected, identified to family or genus, and stored at -80ºC. These specimens have been shipped to the Western Cotton Research Laboratory, where the predator gut content is being assayed with immunologically-based assays that employ pest-specific monoclonal antibodies (MAbs) for the presence of GWSS egg protein using the ELISA by Drs. Hagler, Fournier and Leon (Hagler et al. 2003). These studies will provide direct evidence of predation by generalist predators.

Objective 3 - Xylella

How important are glassy-winged sharpshooter populations in the urban regions as vectors of *Xf* in nearby agricultural areas? First, GWSS population densities have been relatively low in the SJV urban centers, as previously described. Second, GWSS has a relatively low *Xf* transmission efficiency. Together, the low density and poor transmission efficiency would suggest few GWSS would have *Xf* in their mouthparts and play any role in the movement of the pathogen. We tested adult GWSS collected from ornamental plants in Bakersfield and, to our surprise, found *Xf* in GWSS (mouthparts) collected from oleander, Xylosma, and Chinese elm. The positive results do not necessarily mean that the GWSS acquired the *Xf* from the plants that they were collected on as the adults move between host plants often.

How important are GWSS nymphs in the movement of *Xf* among ornamental plants and to vineyards? Nymphs shed the lining of their gut with each molt before adulthood, loosing any *Xf* living there and therefore provide a better indication of acquisition. The initial screening of GWSS nymphs used a "presence" or "absence" of groups of nymphs collected and therefore data are presented as such, rather than a percentage. In the initial collections, *Xf* was found only in GWSS nymphs collected from oleander (in the Bakersfield region). It is also important to note that all GWSS samples testing positive for *Xf* were analyzed for bacterial strain differences and analyses showed that the bacteria present are not of the PD type, but could be oleander, almond, oak, peach or plum. Most likely the *Xf* is oleander strain, which does not pose an immediate threat to nearby vineyards because this strain does not cause PD in grapes

CONCLUSIONS

We have described GWSS population density and age structure on ornamental plants common in residential landscaping in the SJV. We have further described natural enemy presence. This research can be added to information collected in Riverside and Ventura counties to help predict GWSS movement and develop control programs. The research has broader implications for use of ornamental landscape and riparian plants within agricultural settings (e.g., landscaping around farm buildings and homes). Plants which act as preferred hosts for both vector and pathogen can be target for control. By testing GWSS for the presence of *Xf*, researchers will identify potential sources of the pathogen, thereby preventing potential epidemic spread of Pierce's disease causing *Xf* throughout a reservoir of ornamental host plants. To see a list of host plants, for both *Xf* and GWSS) go to: http://nature.berkeley.edu/xylella.

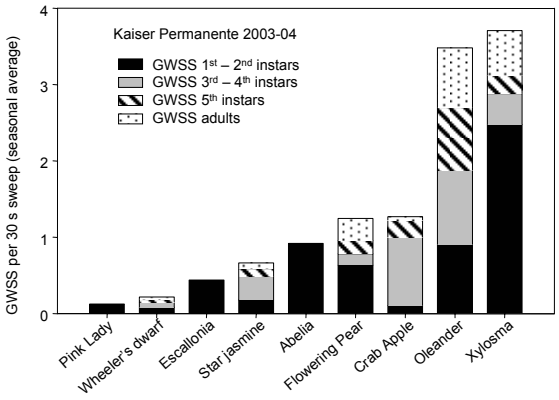

Figure 1. The seasonal average for host plant preference GWSS adults and nymphs was clearly towards oleander and Xylosma at this sampling site. Data of the seasonal average are skewed by the large spring GWSS population density.

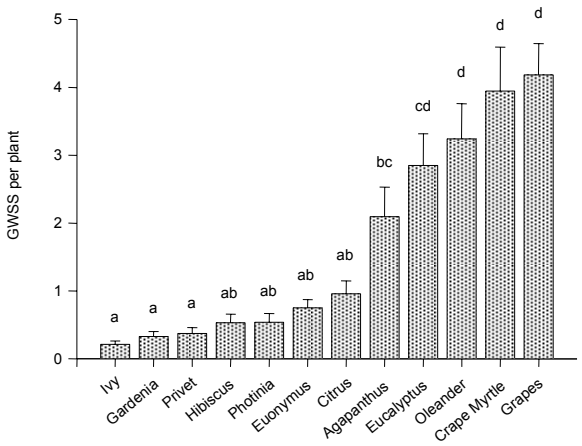

Figure 2. Average densities (± SEM) of GWSS (nymphs and adults) were significantly different among perennial host plants, Tukey's HSD at $P < 0.05$. Data are seasonal averages, and biased towards host species preferred in June and July, when GWSS densities were the highest.

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IDENTIFYING KEY PREDATORS OF THE VARIOUS GLASSY-WINGED SHARPSHOOTER LIFESTAGES

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Reporting Period: The results reported here are from work conducted from November 1, 2003 to October 1, 2004.

ABSTRACT

Glassy-winged sharpshooter (GWSS) egg-specific monoclonal antibody (MAb) and GWSS-specific genetic markers have been developed for use as diagnostic tools for predator gut content analysis. Feeding trials were conducted to determine how long a MAb-based ELISA can detect GWSS remains in the guts of *Chrysoperla carnea* and *Harmonia axyridis*. We found that *C. carnea* can yield positive ELISA reaction for the presence of GWSS egg antigen for up to 24 hours after eating an egg. Further results showed that the detection period of GWSS egg antigen in *H. axyridis* is less than 6 hours. Using mitochondrial COII primers specific to GWSS, we obtained successful amplification of GWSS DNA fragments from *H. axyridi* that consumed six GWSS eggs. Optimization tests are underway to increase the efficacy of GWSS-specific genetic primers to detect pest DNA in predator guts. Feeding trials with additional predators (*Zelus renardii*, *Sinea diadema*, and several spider species) are currently being performed.

INTRODUCTION

Effective control of GWSS will require an areawide integrated pest management approach (AW-IPM). A major component of AW-IPM is the exploitation of the pest's natural enemies, which, when utilized to their greatest potential, can increase the effectiveness of other control tactics. Unfortunately, very little information exists on GWSS's predaceous natural enemies. Evidence of predation of GWSS eggs and adults has been observed in the field (JH pers. obs.); however, the composition of the predator complex, and the relative impact of each predator on GWSS mortality is unknown. A major obstacle is the difficulty of studying predators in their natural environment. Unlike parasitoids, predators rarely leave evidence of attack. Laboratory experiments can be used to evaluate the suitability of particular prey and the rates of predation. However, lab studies seldom translate to field situations. Direct field observations are sometimes used to identify predators of key pests, but the small size and cryptic nature of predators and GWSS make direct observations difficult and laborious. Predator gut content analysis represents a valid approach to investigate predation. Currently, the state-of-the-art predator stomach content assays include enzyme-linked immunosorbant assays (ELISA) for the detection of prey-specific proteins (Hagler 1998; Hagler & Naranjo 1994ab) and polymerase chain reaction (PCR) assays for the detection of prey-specific DNA (Symondson 2002). To this end, we have developed GWSS egg-specific MAbs (Hagler et al. 2002; Fournier et al. submitted) and GWSS-specific primers (de León & Jones 2004). Both assays provide an avenue to qualitatively assess the impact of predator species on GWSS populations.

OBJECTIVES

Our main objective is to identify the composition of the GWSS predator complex using pest-specific ELISA and PCR assays. However, several optimization studies are needed (e.g. detectability half-life) before these assays can be used to examine field-collected predators. Here we report results of laboratory tests on detection periods of GWSS egg antigen in the guts of two generalist predators, the green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) using a GWSS egg-specific ELISA. We also present preliminary results on predator gut content analysis using PCR.

RESULTS:

ELISA Response to Lacewing that Consumed GWSS Eggs

Predators were placed individually in Petri dishes and starved for 36 h. Lacewings were then fed one or two GWSS eggs (within a 30-min time frame) and isolated from food for 0, 6, 9, 12, 24, or 36h at 25°C, photoperiod of 16:8h (L:D), and then frozen (-80ºC). Negative controls were individuals that did not eat any GWSS eggs. Each lacewing was analyzed by indirect

ELISA for the presence of GWSS egg antigen (methods described in Hagler et al. 2002). Data indicate that the number of ELISA positive reactions decreased over time (Table 1). All negative controls yielded negative ELISA absorbance values. Significant differences between the mean absorbance of values of the lacewings fed GWSS eggs and their negative control counterparts was found in all post-feeding time intervals, except for time=24 and 36 h.

Table 1. ELISA results testing for the presence of GWSS egg antigen in the guts of *Chrysoperla carnea* (3rd instar larva).

Treatments [a]	Negative Control			Lacewing fed with GWSS eggs		
	Absorbance at 405 nm, mean ± SD	Critical value[b]	% positive reactions (N) [c]	Absorbance at 405 nm, mean ± SD	% positive reactions (N)	Signifi-cance[d]
0h	0.089±0.003	0.098	0 (15)	0.526±0.488	95 (19)	***
6h	0.072±0.006	0.090	0 (22)	0.176±0.142	62 (21)	***
9h	0.076±0.004	0.088	0 (19)	0.197±0.167	76 (21)	**
12h	0.074±0.007	0.095	0 (21)	0.147±0.149	43 (23)	*
24h	0.077±0.008	0.101	0 (14)	0.170±0.180	36 (22)	N.S.
36h	0.073±0.005	0.088	0 (22)	0.072±0.011	0 (22)	N.S.

[a] post-GWSS egg consumption intervals (hour).
[b] Mean + 3SD of the negative controls (Sutula et al. 1986).
[c] Based on the critical value of the negative control predators. N=total no. of individuals assayed for each treatment.
[d] Significant differences (*t* test) between negative control predators and their counterparts fed GWSS eggs: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; N.S., not significant.

ELISA Response to Multicolored Asian Lady Beetle that Consumed GWSS Eggs

Adult beetles were placed in individual Petri dishes and starved for 36 h. Each adult was fed six GWSS eggs (within a 60-min time frame) and isolated from food for 0 or 6h and then frozen (-80ºC). Negative controls were individuals that did not eat any GWSS eggs. We analyzed the dissected gut of each individual by indirect ELISA for the presence of GWSS egg antigen. All negative controls yielded negative ELISA absorbance values. We found that 65% of the individuals that ate GWSS eggs scored positive at time=0 h, and 8% at time=6h. A significant difference between the mean absorbance values of the beetles fed GWSS eggs and their negative control counterparts only occurred for the time=0h treatment.

Predator Gut Content Analysis Using PCR Assays

We are currently optimizing a PCR assay to detect GWSS DNA in the guts of various species of predators. Several pairs of primers were designed to amplify GWSS-specific fragments from: (1) randomly amplified polymorphic DNA (RAPD) based on sequence characterized amplified regions (SCAR); and (2) the mitochondrial cytochrome oxydase subunit I (COI) and subunit II (COII) genes (de León & Jones 2004). The size of amplified fragments of GWSS DNA varies from 166 to 302 bp. Adult *H. axyridis* fed six GWSS eggs were immediately frozen (-80ºC) after eating. Negative controls were beetles that did not eat any GWSS eggs. Each individual was homogenized in a lysis buffer solution, DNA was extracted using a DNeasy kit (Qiagen Inc., Valencia CA) and subjected to PCR using GWSS-specific COII primers. GWSS DNA was successfully amplified from *H. axyridis* extracts (Figure 1). Further tests are underway comparing the efficacy of different primer sets and determining the half-life detection interval of GWSS DNA in the guts of several predator species (*C. carnea*, *Z. renardii*, *S. diadema*, and several species of spiders).

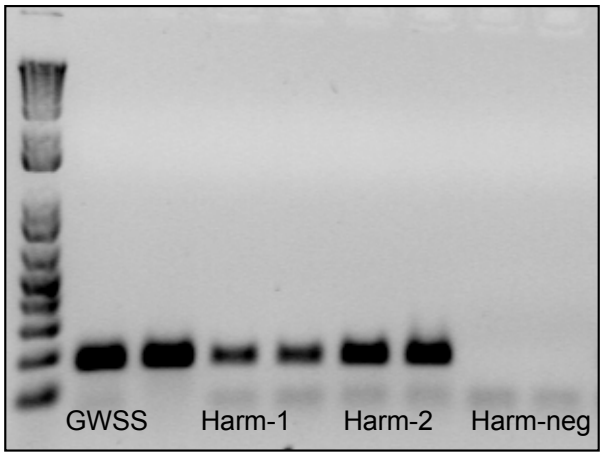

Figure 1. PCR assays were performed using GWSS-specific COII primers on *Harmonia axyridis*. This 2% agarose gel shows that GWSS DNA fragment (178bp) was amplified from the following samples (duplicates): positive control (GWSS), predators fed six GWSS eggs (Harm-1, Harm-2). No amplification occurred for the *H. axyridis* individual that did not consumed any GWSS eggs (Harm-neg).

CONCLUSIONS

We showed that molecular gut content assays can be used to detect GWSS remains in the guts of predators. Once optimization tests are complete we will assay extensive numbers of field-collected predators. We will be able to distinguish specimens that preyed upon immature and adult life stages of the GWSS via the PCR assay and those that consumed eggs via the ELISA assay. An understanding of the key natural enemies of GWSS will contribute to an areawide IPM approach for GWSS control. Once key predators are identified they can be better exploited for conservation and augmentative biological control programs.

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FUNDING AGENCIES

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ULTRASTRUCTURAL CONTRIBUTIONS TO THE STUDY OF THE GLASSY-WINGED SHARPSHOOTER AND PIERCE'S DISEASE

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Reporting Period: The results reported here are from work conducted from October 2003 to October 2004.

ABSTRACT
A variety of microscopic techniques including light microscopy, confocal scanning light microscopy, transmission electron microscopy, and scanning electron microscopy are helping to elucidate the structure and function of the mouthparts and the salivary sheath of the glassy-winged sharpshooter, a vector of Pierce's disease.

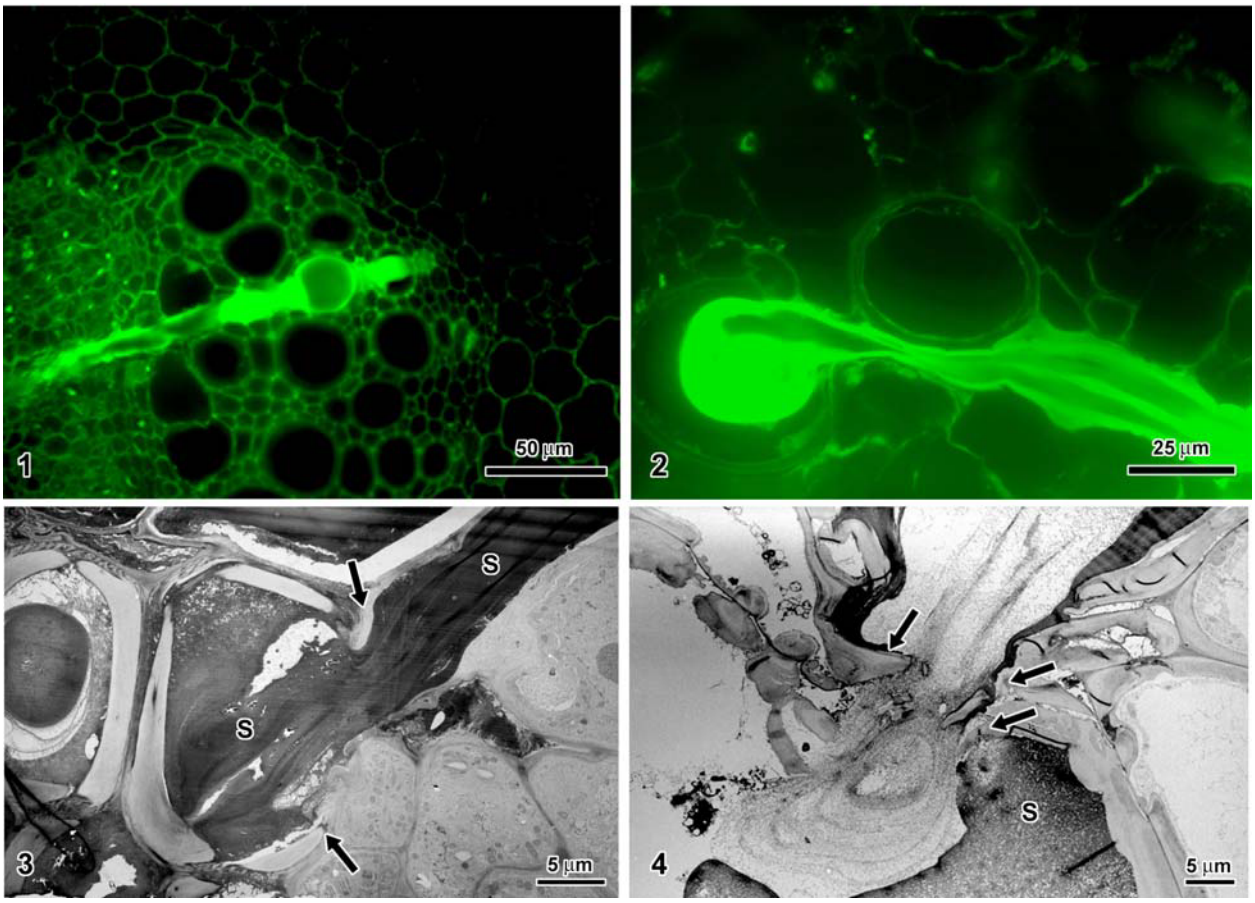
OBJECTIVES
1. Describe the morphology and ultrastructure of the glassy-winged sharpshooter mouthparts.
2. Describe stylet penetration and the function of each stylet pair during feeding.
3. Ascertain the path of mouthparts from the epidermal layer to the vascular tissue of the host plant, and to ascertain if the sharpshooter has fed in parenchymatous or phloem tissue en route to xylem tissue.
4. Determine the ultrastructure of the salivary sheath and its association with all plant tissues encountered from the epidermal layer to the xylem tissue.

RESULTS AND CONCLUSIONS
The glassy-winged sharpshooter (GWSS) has a significant economic impact as the vector for the transmission of *Xylella fastidios*a, which causes Pierce's disease in grapes, leaf scorch in oleander and almonds, and variegated chlorosis in citrus. Different strains of the bacterium also cause diseases of avocados, peaches, plums, apricot, cherries, and many other trees and ornamentals (Purcell and Saunders 1999, Purcell et al. 1999). The GWSS feeds primarily on the xylem fluid of more than 100 different host plants from more than 35 plant families.

In response to the tremendous economic importance of this insect, a variety of research avenues are under investigation to develop control or management strategies. One important research area that has not received adequate attention is the interaction between the GWSS and the host plants. Until very recently we knew very little regarding the structure of the GWSS mouthparts, and simply assumed that they were similar to those of other leafhoppers. During the last two years, we have provided extensive ultrastructural descriptions of the GWSS mouthparts, including several new sensory structures associated with the sharpshooter stylets and labium (Leopold et al. 2003, Freeman et al. 2002, 2003).

Many unbranched salivary sheaths and branches of very complex sheaths, formed by nymph and adult sharpshooters, do not always extend directly from the host-plant epidermis to the xylem tissue. GWSS stylets may penetrate only as far as the vessel element wall or they may actually fragment the lignified wall and enter the cell lumen (Figures 1-4). Several vessel elements in a vascular bundle or secondary xylem may be damaged during a single sharpshooter probe (Figure 1). Fragmented vessel elements (Figures 2-4) would change the dynamics of water translocation. Penetrated vessel elements are only infrequently surrounded by salivary sheath material, which raises questions as to the function of the sheath in reducing or preventing cavitation. Penetrated vessel elements can, however, become partially or completely occluded with GWSS salivary sheath material (Figures 1-3), a situation that would also disrupt water translocation even in the absence of *X. fastidiosa*.

The glassy-winged sharpshooter ingests large volumes of xylem fluid during feeding, most of which is quickly excreted. We have noted that both nymph and adult sharpshooters produce exudates during probes that do not reach the xylem, suggesting that they may be feeding in host cells located between the epidermal layer and the xylem. The transfer of *Xylella* to parenchyma cells outside of the xylem (Backus et al. 2003) might be another indicator that sharpshooters are feeding in non-xylem tissues. With a high assimilation efficiency of carbon (Brodbeck et al. 1993, 1995, 1996), there may be a nutritive advantage for even limited feeding in parenchymatous tissues. We now have preliminary data showing that first, second, and third-instar nymphs successfully feed on sunflower stems where the xylem is located too distant from the epidermis to be reached by the length of their stylets. We note that less than 50% of first and second instars have salivary sheaths terminating in the xylem even when the xylem is within the reach of their stylets. Third and fourth instars are only slightly more successful.



Figures 1, 2. Confocal scanning light micrographs. Figure 1. Several vessel elements damaged by a single GWSS stylet probe.

Figure 2. Salivary sheath material occluding a fragmented vessel element

Figures 3, 4. Transmission electron micrographs showing fragmented vessel element walls (arrows) and salivary sheath occlusions (s).

In our greenhouse and laboratory studies, host plants fed on by sharpshooters for several days to weeks begin to show symptoms similar to those of plants infected with the bacterium *X. fastidiosa.* These symptoms occur in our host plants even though the sharpshooters we are studying are free of *Xylella.* Previous reports indicated that the symptoms of Pierce's disease may occur very shortly after inoculation with *X. fastidiosa*, long before there is a significant increase in the population of the bacteria to a level believed necessary to produce symptoms (Labavitch *et al.* 2002). Many plant species infected by strains of *X. fastidiosa* show no symptoms of Pierce's disease (Purcell and Saunders 1999). Our research is ongoing to determine the correlation of mechanical damage and occlusion of vessel elements to the onset of symptoms in non-infected host plants

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FUNDING AGENCIES

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EPIDEMIOLOGY OF PIERCE'S DISEASE IN THE CENTRAL SAN JOAQUIN VALLEY OF CALIFORNIA: FACTORS AFFECTING PATHOGEN DISTRIBUTION AND MOVEMENT

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Reporting Period: The results reported here are from work conducted November 2003 to September 2004.

ABSTRACT
The primary objective of this research was to characterize the seasonal abundance, dispersal, and overwintering biology of the glassy-winged sharpshooter (GWSS), a primary vector of *Xylella fastidiosa (Xf*). Moreover, to identify where the vector(s) acquire the pathogen, to determine when vectors move into vineyards and transmit the pathogen to grapes, and to genetically characterize the populations of *Xf* isolated from GWSS collected in different perennial cultivated and non-cultivated plant species. Based on results of seasonal plant utilization by GWSS in our study through the winter of 2003-04 and into the subsequent growing season, we conclude that host plant species can significantly influence GWSS population biology. GWSS adult, nymph, and egg mass densities varied among perennial, cultivated crop plant species and non-cultivated weed species examined in this study. Perennial crop species examined included sweet cherry, navel, lemon, olive, avocado, peach, plum, pomegranate, pistachio, and grape. Adult GWSS dispersed into and fed upon a wide range of these crop species with the largest dispersing populations observed in citrus (lemon and navel) and pomegranate, similar to our findings in 2003. Adult GWSS were also regularly collected from and observed feeding upon a wide range of non-crop weed species within and surrounding experimental orchard crops. Nymph populations were not equally represented across all perennial tree crops with increased populations collected from citrus, pomegranate, and also non-crop annual weed species. Overwintering adult GWSS were consistently collected in relatively low population densities on citrus, pomegranate, avocado, plum, peach, and non-crop annual weed species. Patterns of adult GWSS capture among the distances sampled along linear transects extending into perennial crops were dissimilar among perennial crops. The presence of *Xf* in a subsample of vectors collected from different perennial crops and on non-crop species is underway using a multiplex PCR protocol to differentiate genomic populations.

INTRODUCTION
The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, was introduced into Southern California in the late 1980's and later identified in 1994 (Blua et al. 1999). The insect regularly occurs in most of Southern California and has become established along eastern portions of the San Joaquin Valley of central California. Large populations of the GWSS are becoming widely distributed and will reportedly feed and oviposit on a wide range of perennial crop and ornamental plant species as well as numerous non-crop wild plant species (Adlerz and Hopkins 1979, Daane and Johnson 2003). This sharpshooter has continued to expand its range in the state and is expected to affect the overall increase in plant diseases caused by *Xylella fastidiosa* (*Xf*) (Purcell and Saunders 1999a). Strains of *Xf* have a complex pathogenic relationship with a diverse host range including members of both monocots and dicots (Chen et al. 2000). Analyses of the genetic diversity of *Xf* have begun to elucidate differences between many of the strains (Chen et al 1995, Hendson et al. 2001, Pooler and Hartung 1995). Knowledge of the genetic diversity of strains that comprise the population of *Xf* in the central San Joaquin Valley (SJV) of CA, especially as it relates to insect vectors, will help in devising effective strategies for managing Pierce's disease (PD), as well as other diseases caused by this bacterium.

Xylella fastidiosa is transmitted by xylem feeding sharpshooters (Cicadellidae) and spittlebugs (Cercopidae) (Hill and Purcell 1997, Purcell and Frazier 1985). In California, there are at least 20 species capable of transmitting the pathogen, although only four species are considered to be epidemiologically important in grapes (Pearson and Goheen 1988). Based on the population dynamics of native sharpshooter species in coastal California vineyards, much of the spread of *Xf*, especially early in the season when it is most damaging to grapevines, are by adults that move into the vineyard from outside host sources (Purcell and Saunders 1999b). Knowledge of which vector species transmit *Xf* in the central SJV, where they acquire the

pathogen, when they move into vineyards, and when they spread the pathogen to grapes is critical to understanding and managing the spread of PD in this area.

OBJECTIVES

1. To identify and characterize the seasonal abundance of the primary vectors of *Xf* and seasonal patterns of insect dispersal.
2. Compare the genetic structure of *Xf* strains isolated from GWSS collected from perennial, cultivated and non-cultivated plant species.

RESULTS

Objective 1

Examination of the seasonal host utilization patterns and dispersal biology of the glassy-winged sharpshooter, *Homalodisca coagulata* (GWSS) within and among a variety of perennial crop plant species has been monitored through the winter (2003-04) and following spring and summer seasons of 2004. Experimental sites are located in GWSS-infested areas of Tulare County, California. The results of these studies continue to provide valuable insight into the relative importance of different crop types as predominant overwintering habitats, ovipositional substrates, and preferred feeding hosts for GWSS. Patterns of crop utilization were monitored within perennial crop species including grape, citrus (navel and lemon), stonefruit (sweet cherry, peach, and plum), olive, and avocado at each of three locations for each crop type. Additionally, non-crop weed vegetation was monitored throughout the season at three experimental sites along with riparian vegetation. Host utilization was assessed monthly at each of three locations for each crop type based on sweep/beat-net sampling for adult and immature GWSS and visual inspections for GWSS egg masses. Results from our second year again indicate that host plant species influences GWSS population biology. Similar to our findings in 2003, the largest mean number of adult GWSS were collected from citrus (navel and lemon) and pomegranate whereas mean nymphal population densities were lower than the previous season. More nymphs were present in navel orange and pomegranate with fewer nymphs collected in olive, avocado, cherry, plum, and peach. Non-crop plant species upon which adult and nymphal GWSS were collected included red-root pigweed, prickly lettuce, annual sowthistle, little mallow, lambsquarters, field bindweed, blue morning glory, curly dock, evening primrose, johnsongrass, and ground cherry. The greatest mean number of GWSS egg masses were collected from both citrus and pomegranate.

Seasonal dispersal of adult GWSS was again monitored within and among the previously indicated perennial crop plant species Traps were suspended 2 m above the ground between tree canopies along 4 linear transects at each of 3 experimental locations for each crop sampled. Beginning November 2003, a total of 11,677 adult GWSS, 29 green sharpshooters (GSS, *Draeculacephala minerva*), and 351 spittlebugs (Cercopidae) were captured on yellow sticky cards. Temporal patterns of GWSS capture were similar in citrus and pomegranate throughout the 2004 sampling season representing dispersal of both overwintered and 1st generation adult GWSS. Seasonal patterns of GWSS capture in olive, avocado, and plum was dissimilar to that of either citrus or pomegranate similar to the patterns observed in 2003. Beginning November 2003, we have begun to closely monitor the overwintering host utilization patterns of adult GWSS among the variety of perennial crop and non-crop weed species previously listed. Overwintering adult GWSS have been sampled monthly (Nov – Feb, 2003) in perennial tree crops by beating/shaking all scaffolds over two, 80 ft^2 white, PVC tarps that flank both sides of the tree stem and in non-crop weed species using sweep net collections described previously. Adult GWSS have been collected overwintering on citrus (lemon and navel), pomegranate, peach, plum, and avocado averaging 0.2, 0.4, 0.9, 0.02, 0.05, and 0.5 adult GWSS/tree, respectively, over the four month sample interval. Mean populations of adult GWSS swept from non-crop annual vegetation have averaged 1.1, 2.4, 0.9, and 0.3 adult GWSS/50-sweep sample over the four month sample interval, respectively. To examine the seasonal population biology of GWSS utilizing non-crop host species, GWSS, native sharpshooters, and all spittlebugs have been sampled monthly from the ground cover and surrounding vegetation at each of the 3 experimental locations with high populations of GWSS present in 2003. At each location, sharpshooter and spittlebug adults and nymphs associated with the ground cover and surrounding non-crop vegetation are sampled using a standard sweep net (100 sweeps at each of 10 sites per location for ground cover).

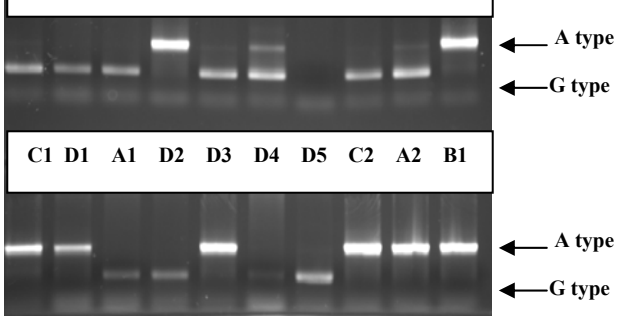

Figure 1. Polymerase chain reaction products from 18 *Xf* isolations collected in 2003 illustrating 2 genotypes (A = almond; G = grape).

Objective 2

The presence of *Xf* in a subsample of vectors captured among the different perennial crops and on non-crop species has begun using PCR. Genomic DNA is first isolated and initially screened against RST 31/33 universal primers to detect all *Xf* strains. The diversity of the chosen *Xf* isolates will be assessed using RAPD-based protocols and single nucleotide polymorphisms (SNPs) from genome loci of taxonomic importance deduced from the available genome sequences. Previous studies have demonstrated that these

protocols generate sufficient polymorphisms within *Xf* to enable grouping of strains according to host associations. SNP analyses represent one of the most recent technologies used for comparative studies of closely related bacteria. Based on published genomic information, strain specific primers recently will be used to investigate the pathotype profile using the 16S rDNA intergenic region. Results from our current season's research indicate that this multiplex PCR protocol can differentiate genomic populations which might co-exist in infectious vectors (Fig. 1). Here again, attempts will also be made to quantify *Xf* in selected insect vectors to identify the population dynamics of *Xf* within a vector population.

CONCLUSIONS

The results obtained from the second year of this project remains consistent with our first year observations and has generated significant new information regarding the seasonal host utilization patterns, dispersal, and overwintering biology of GWSS in the central SJV of California. This information will improve our understanding of the epidemiology of Pierce's disease which will also be useful in understanding the epidemiology of other economically important diseases caused by *Xf* for which GWSS may become an important vector. This objective directly addresses gaps in our present understanding that must be filled in order to develop comprehensive PD and GWSS management strategies. This research has expanded on previous work by documenting important aspects of the population biology of GWSS in the agricultural landscape of the central San Joaquin Valley of California. An improved knowledge of the genetic diversity of strains that comprise the population of *Xf* detected from potentially infectious GWSS will further help in devising effective strategies for managing Pierce's Disease, as well as other important diseases caused by this bacterium.

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A NOVEL IMMUNOLOGICAL APPROACH FOR QUANTIFYING PREDATION RATES ON GLASSY-WINGED SHARPSHOOTER

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ABSTRACT
A glassy-winged sharpshooter (GWSS) protein marking system is being developed for use as a diagnostic tool for predator gut content analysis. We determined that GWSS can be marked with 100% efficiency for at least 7 days after feeding on protein-marked plant material or spraying with a topical protein solution. Moreover, feeding trials have shown that protein marked insects can be detected by a protein-specific ELISA in the guts of predators that consumed them. Field studies are being initiated that will quantify the predation rates of an assemblage of predators on GWSS using a multitude of protein-specific ELISAs.

INTRODUCTION
Very little information exists on predaceous natural enemies of GWSS. While predaceous arthropods are important regulators of arthropod populations (Luff, 1983; Sabelis, 1992; Symondson et al., 2002); identifying the feeding choices and amount of prey consumed by generalist predators is very difficult. Predators and GWSS are small, elusive, cryptic (Hagler et al., 1991), and the predators may feed exclusively at night (Pfannenstiel & Yeargan, 2002). Hence, visual field observations of predation are extraordinarily difficult to obtain. Moreover, predators do not leave evidence of attack. Perhaps the most frequently used experimental approach for evaluating natural enemies in the field are through studies conducted in field cages (Luck et al., 1988). Such studies require manipulation of either the natural enemy or the targeted prey population(s) within the cage (e.g., the removal or introduction of the organism of interest). Mortality of the pest can be estimated based on the presence or absence of the pest (Smith & De Bach, 1942; Leigh & Gonzalez, 1976; Luck et al., 1988; Lang, 2003). Such studies have documented the qualitative impact of manipulated predator assemblages on many types of pests, but they do not provide quantitative information on predation rates or evidence of which predator in the assemblage is exerting the greatest biological control. Often the only direct evidence of arthropod predation can be found in the stomach contents of predators. Currently, the state-of-the-art predator stomach content assays include enzyme-linked immunosorbent assays (ELISA) for the detection of pest-specific proteins (Hagler, 1998) and PCR assays for the detection of pest-specific DNA (Agustí et al.; 1999; Symondson, 2002; Greenstone & Shufran, 2003).

ELISAs have been widely used to identify key predators of certain pests, including GWSS (Ragsdale et al., 1981; Sunderland et al., 1987, Hagler et al., 1992, 1993, 1994; Hagler & Naranjo, 1994ab; Bacher et al., 1999; Fournier et al., in prep). The simplicity and low cost of conducting an ELISA lends itself to the efficient screening of hundreds of field-collected predators per day. However, polyclonal antibody-based ELISAs often lack species specificity and monoclonal antibody-based ELISAs are too technically difficult, costly, and time consuming to develop for wide scale appeal (Greenstone, 1996). Moreover, pest-specific ELISAs share the same limitation as the other predator evaluation methods; the quantification of predation rates is impossible (see Hagler & Naranjo, 1996; Naranjo & Hagler, 1998 for reviews). PCR assays using pest-specific DNA probes might be less expensive to develop (Greenstone & Shufran, 2003), but PCR assays are also not quantifiable and they are more costly, technical, tedious, and time consuming to conduct than ELISAs (pers. obs.).

Due to the reasons discussed above, quantifying predation rates is extremely difficult. These difficulties have resulted in a dearth of information on the quantitative impact that generalist predators have on suppressing pest populations. The many shortcomings of each method of predator assessment described above were the impetus for us to develop a technique to quantify predator activity. The technique combines our previous research using pest-specific MAb-based ELISAs to detect predation (Hagler et al., 1991, 1993, 1994, 2003) with protein marking ELISAs we developed to study arthropod dispersal (Hagler & Miller, 2002; Hagler, 1997a, b; Hagler & Naranjo, 2004; Hagler & Jackson, 1998; Hagler et al., 2002). Here we describe a technique for marking individual GWSSs, each with a unique protein. In turn, the gut contents of each predator in the assemblage can be examined by a multitude of protein-specific ELISAs to determine how many GWSS were consumed and which predator species consumed them. The advantages of immunomarking prey over prey-specific ELISAs are: (1) prey-specific antibodies (or PCR probes) do not need to be developed, (2) the protein-specific sandwich ELISAs are more sensitive than the indirect prey-specific ELISAs (Hagler et al., 1997), (3) a wide variety of highly specific protein/antibody complexes are available, (4) the specificity of each antibody to its target protein facilitates the marking and examination of

the gut contents of every predator in the assemblage by a myriad of protein-specific ELISAs, and (5) all of the proteins and their complimentary antibodies are commercially available at an affordable price.

OBJECTIVES

We are in the preliminary phase of a research project dedicated to quantifying predation rates on GWSS nymphs and adults and qualifying predation on eggs. There are enough protein/antibody complexes commercially available that each GWSS in a field cage can be marked with a specific protein. We will mark individuals (e.g. adults and nymphs) and release them for 6 hours into a cage containing an assemblage of predators. The experiment will contain a day and night treatment. Observed mortality for each GWSS life stage will be determined by simply counting the number of GWSSs remaining in each cage. Each predator will then be examined by a multitude of protein-specific ELISAs to determine which predators ate GWSS nymphs and adults and how many each predator consumed. Then, each predator will be examined by a GWSS egg-specific ELISA to determine the frequency of predation on GWSS eggs (see Fournier et al. in this volume). Specifically, this study will: (1) quantify predation on GWSS nymphs and adults, (2) qualify predation on GWSS eggs, and (3) determine the circadian feeding activity of predators. Results obtained from this research will enhance our basic understanding of predator-prey interactions and aid in evaluating the efficacy of generalist predators for a conservation biological control program or an inundative biological control program.

RESULTS

We (JRH) conducted feasibility studies to determine if protein markers can be substituted for pest-specific MAbs for the immunological detection of prey in predator guts. In a series of lab studies, we fed a wide variety of predators (e.g., chewing and piercing/sucking type predators) both large and small prey marked with rabbit immunoglobulin G (IgG). In turn, the gut contents of each predator was analyzed by a rabbit IgG-specific ELISA. The results showed that, regardless of the predator species and the size of prey consumed, the rabbit IgG ELISA could easily detect the mark in the predator's stomach for at least 6 hours after feeding (Figure 1).

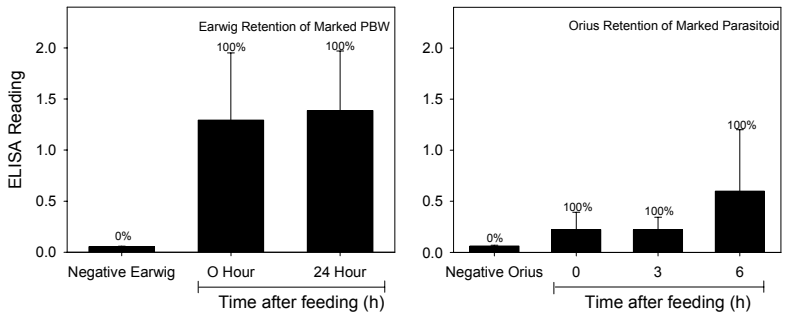

Figure 1. Mean (±SD) ELISA readings for the retention of rabbit IgG in the gut of two types of predators that consumed either a single 2nd instar pink bollworm larva or an adult parasitoid (*Eretmocerus emiratus*) marked with 5.0 mg/mL of rabbit IgG. The numbers above the error bars are the percentage of individuals positive for rabbit IgG. The negative predators consumed unmarked prey. Note: these data were chosen for display because they represent the extreme case scenarios (e.g., a large chewing predator eating a relatively large marked prey and a small piercing/sucking predator eating a very small marked prey). Similar studies are being conducted on GWSS.

The next study was designed to determine if we could mark adult GWSSs. In a pilot study, we marked (internally and externally) adult GWSS with rabbit IgG protein using the techniques described below.

Internal Marking

GWSSs were provided a chrysanthemum (mum) that was previously marked with a topical spray of a 5.0 mg/mL rabbit IgG solution. Individuals were allowed to feed on a protein-marked mum for 48 h. The GWSSs were removed from the protein-marked mum and placed on unmarked mums for 3, 5, or 7 days after marking and then analyzed for the presences of rabbit IgG by the anti-rabbit IgG ELISA described by Hagler (1997a). The efficacy of the marking procedure is given in Figure 2.

External Marking

We applied an external mark to individual GWSSs by spraying them with 1.0 ml of a 0.5 mg/mL rabbit IgG solution using a medical nebulizer (Hagler 1997b). The GWSS were air-dried for 1 h and then placed on mums for 3, 5, or 7 days after marking and then analyzed for the presence of rabbit IgG by ELISA. The efficacy of the marking procedure is given in Figure 2.

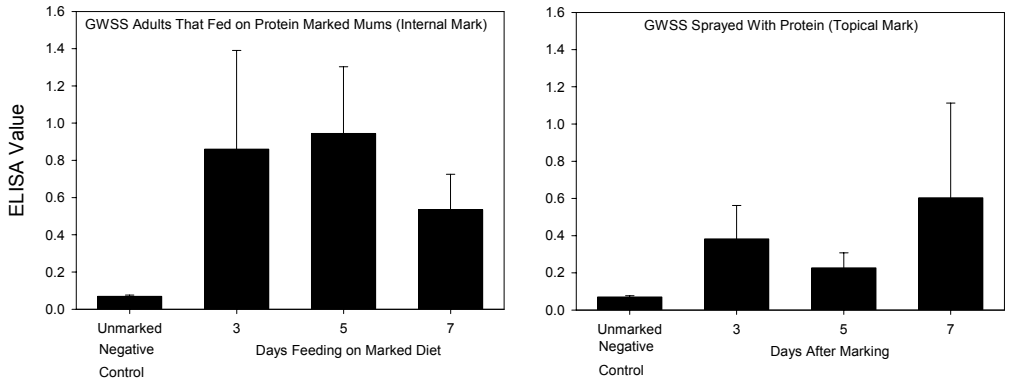

Figure 2. The efficacy of the internal (left graph) and external marking procedure (right graph) (n=8 to 16 per treatment). All of the GWSSs assayed 3, 5, and 7 days after marking yielded positive ELISA responses for the presence of rabbit IgG. All of the unmarked GWSSs yielded negative ELISA responses.

Results indicate that the protein marking procedure works for at least 7 days after marking GWSS. The next phase of our research (in progress) will be to mark individual GWSSs using the methods described above. Specifically, 10 individual GWSSs will be marked, each with a unique protein (see Table 1). The 10 GWSSs will then be placed in a field cage containing various predator species. The predator assemblage examined will represent those predators commonly found in areas inhabited by GWSS (JRH, pers. obs.). A partial list of the predator assemblage that will be examined and their probable feeding behaviors is given in Table 2. After 6 h in the cage, every remaining predator will be collected and analyzed by 10 different protein-specific ELISAs. A hypothetical example of the data we will generate over the next year is given in Table 3.

Table 1. A listing of the proteins that will be used to mark 10 individual GWSS.

Individual GWSS	Protein marker
1	Rabbit IgG
2	Guinea pig IgG
3	Equine IgG
4	Mouse IgG
5	Dog IgG
6	Pig IgG
7	Bovine IgG
8	Cat IgG
9	Rat IgG
10	Sheep IgG

Table 2. A listing of the arthropod assemblage to be examined.

Species	Stage[1]	Classification[2]	Likely GWSS prey[3]
H. convergens	Adult/immature	Carnivore	Egg
Zelus renardii	Adult/immature	Carnivore	Nymph/Adult
Geocoris punctipes	Adult	Omnivore	Egg/early instar nymph
Spiders Salticidae Clubionidae Agelenidae Araneidae	Adult/immature	Carnivore	Nymph/Adult
Earwig	Adult/immature	Omnivore	Egg, nymph, adult
Chrysoperla carnea	Immature	Carnivore	Egg
Preying mantis	Adult/immature	Carnivore	Nymph, adult
Syrphid fly	Immature	Carnivore	Egg
Coccinella septempunctata	Adult/immature	Carnivore	Egg

[1/]The predator life stage that will be examined.
[2/]The primary feeding habit of each species.
[3/]The most likely GWSS life stage that will be attacked.

Table 3. A hypothetical example of results yielded from a multitude of IgG-specific gut content ELISAs conducted on an individual predator (e.g., *Zelus renardii*). The number of positives yielded in all the assays indicates the number of prey consumed by this single predator.

Predator	Targeted GWSS	Protein marker designated in Table 1	Protein-Specific ELISA	ELISA result[1/]
Z. renardii	1	Rabbit IgG	Anti-Rabbit IgG	-
	2	Guinea pig IgG	Anti-Guinea pig IgG	-
	3	Equine IgG	Anti-Equine IgG	-
	4	Mouse IgG	Anti-Mouse IgG	-
	5	Dog IgG	Anti-Dog IgG	-
	6	Pig IgG	Anti-Pig IgG	+
	7	Bovine IgG	Anti-Bovine IgG	-
	8	Cat IgG	Anti-Cat IgG	+
	9	Rat IgG	Anti-Rat IgG	-
	10	Sheep IgG	Sheep IgG	-

[1/]This individual predator scored positive in the anti-pig and anti-cat ELISAs; therefore it consumed 2 marked GWSSs.

CONCLUSIONS

Although it is widely accepted that predators play a role in pest regulation, we still have an inadequate understanding of, and ability to predict their impact in cropping systems. Frequently parasitoids are given major credit for suppressing pest populations; however, the impact that predators have on suppressing GWSS populations goes unrealized due to the difficulties of assessing arthropod predation as discussed above. The prey marking technique described here circumvents many of the shortcomings of the current methods used to study predation. The preliminary studies described here prove that prey marking can be a powerful method for the immunological detection of predation and can be used to study various aspects of predator feeding behavior. Over the next 2 years we plan to quantify predation rates on GWSS. Ultimately, this information can be used to improve the efficacy of conservation and inundative biological control of GWSS. This research is designed to determine which predators are exerting the greatest biological control on GWSS eggs, nymphs and adults. This information can then be used to develop a comprehensive biological control program that better conserves the populations of those predators exerting the greatest control on the various GWSS life stages.

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IDENTIFICATION OF THE NATIVE PARASITOID FAUNA ASSOCIATED WITH *GRAPHOCEPHALA ATROPUNCTATA* AND HOST SPECIFICITY TESTING OF *GONATOCERUS ASHMEADI* ON *HOMALODISCA LITURATA*

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Reporting Period: The results reported here are from work conducted from May 2003 to October 2004

ABSTRACT
To determine the oviposition preference of female blue-green sharpshooters (BGSS), *Graphocephala atropunctata* (Signoret) (Hemiptera: Cicadellidae), a survey was conducted on southern California wild grape, *Vitis californica* Benth (Vitaceae) growing near Temecula, California in August 2003 where populations of BGSS were known to occur. Female BGSS oviposited into new growth, primarily the succulent tendrils and stems. The under sides of small leaves and petioles were also used for oviposition, but to a lesser extent. Mature stems, large and medium sized leaves and petioles were not utilized for oviposition. Two parasitoids, *Gonatocerus latipennis* Girault and a *Polynema* sp. (Hymenoptera: Mymaridae) were reared from BGSS eggs. Literature reviews revealed a deficiency of known natural enemies for *G. atropunctata*. A sentinel plant study was conducted to further confirm the parasitization of BGSS eggs by these parasitoids. Collectively the *Polynema* sp. and *Gonatocerus latipennis* constitute the first documented parasitic natural enemies of BGSS eggs. A further examination, commencing in January 2004, of the activity of BGSS and its parasitoids in southern California is currently underway. Blue-green sharpshooter adult activity reached its peak in July while bi-weekly samples of wild grape canes and tendrils revealed peak emergence of blue-green nymphs and parasitoids occurred from mid-July to mid-August. No-choice tests with *Gonatocerus ashmeadi* Girault, a parasitoid of the galssy-winged sharpshooter, *Homalodisca coagulata*, and BGSS eggs as part of a non-target impact assessment have yielded few results thus far. However, no-choice tests with *G. ashmeadi* and the native smoke-tree sharpshooter (STSS), *Homalodisca liturata* Ball, yielded no significant differences in percent parasitism of eggs when compared to the GWSS control.

INTRODUCTION
The native BGSS has been a threat to California grape growers for nearly a century due to its excellent transmission efficiency (Hill and Purcell 1995) of the bacterium that causes Pierce's Disease, a severe malady of commercially grown grapes. While much research has been devoted to epidemiologically related issues concerning this insect, little has been done to examine some of the most fundamental life history traits of this native pest, specifically oviposition preference (Severin 1949) and the native Californian parasitoids attacking the eggs of this pest. Further, we intend to investigate possible non-target effects of the exotic egg parasitoids that have been released to control another hemipteran pest, the GWSS, on BGSS and other native California sharpshooters and to identify the native parasitoid fauna associated with these native sharpshooter species. To address these issues, we need to know the oviposition preferences of native sharpshooters associated with particular host plants and their respective natural enemy fauna attacking oviposited eggs. The studies outlined below have determined the oviposition preferences of BGSS on wild grape, have documented its associated egg parasitoids, and provide data on host specificity of *G. ashmeadi,* a parasitoid being used as part of the classical biological control program against GWSS on the targets congener, the native STSS.

OBJECTIVES
1. Classify the native egg parasitoid fauna in California associated with sharpshooters native to California, primarily the smoke-tree sharpshooter (STSS): *Homalodisca liturata* Ball (Hemiptera: Clypeorrhyncha: Cicadellidae: Cicadellinae: Proconiini), blue-green sharpshooter (BGSS): *Graphocephala atropunctata* (Signoret), red-headed sharpshooter (RHSS): *Xyphon fulgida* (Nottingham), and green sharpshooter (GSS): *Draeculocephala minerva* Ball (the latter three, all Hemiptera: Clypeorrhyncha: Cicadellidae: Cicadellinae: Cicadellini).
2. Assess the possible non-target impacts of *Gonatocerus ashmeadi*, *G. trigutattus*, and *G. fasciatus*, parasitioids being used for the classical biological control of GWSS, on the above mentioned native sharpshooters.

RESULTS:

Oviposition Survey

Wild grape plant material collected on 5 August 2003 consisted of: 50 canes (terminal 25 cm of cane), 50 tendrils, 100 large, 100 medium, and 100 small leaves with petioles. The tendrils and small leaves with petioles were selected from the terminal 25 cm sections of the canes. Each of the 50 canes was cut into thirds: upper, middle and lower. No insects emerged from large or medium leaves and their petioles and are thus excluded from further discussion. A total of 49 insects (26 *G. atropunctata*, 18 *Polynema* sp. and five *G. latipennis* parasitoids, Figures. 1 and 2) emerged from plant material collected. The highest percentage of BGSS nymph emergence (18%) occurred in the apical-most portion of the stem, with less emerging from tendrils (14%), and middle (10%) and lower (2%) stems, respectively. A very small percentage of *G. atropunctata* nymphs emerged from small leaves and their petioles. For the parasitoids the highest percent emergence occurred from the tendrils (38%). Collectively, the tendrils and stems yielded the greatest emergence (Figure 3).

G. latipennis

Polynema sp.

Figures. 1 and 2. Parasitoids of the BGSS.

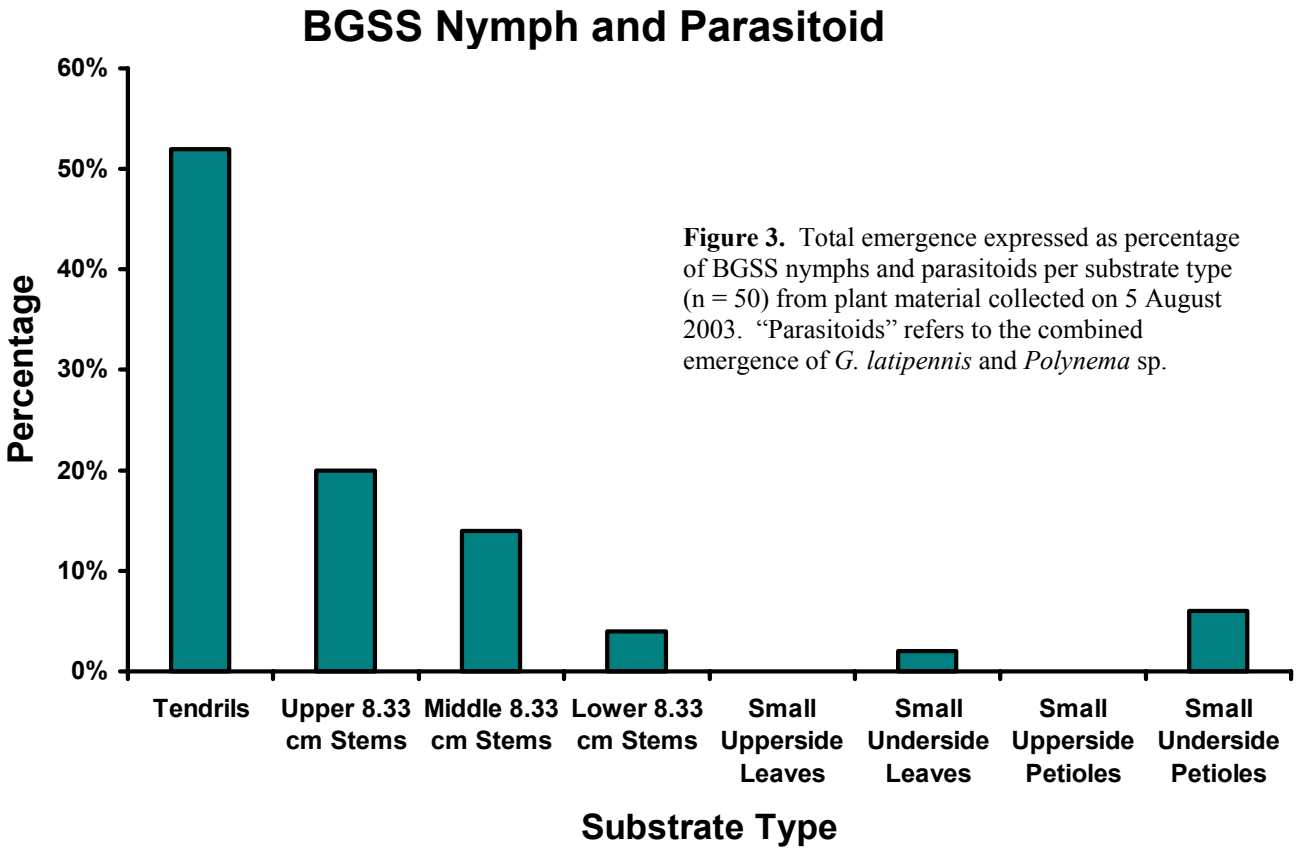

Figure 3. Total emergence expressed as percentage of BGSS nymphs and parasitoids per substrate type (n = 50) from plant material collected on 5 August 2003. "Parasitoids" refers to the combined emergence of *G. latipennis* and *Polynema* sp.

Ten entire grape canes were sampled on 14 August 2003 to account for any possible oviposition substrate not sampled in the previous survey. These canes were cut into thirds (apical, middle and basal), then placed into 10 cm of water in a Mason jar which left approximately 25 cm of cane exposed for emergence of nymphs and parasitoids. Canes and mason jars were then placed into three separate cages, according to their stem position. Cane sections were examined daily for emergence. In total, two BGSS nymphs and 16 *Polynema* sp. emerged from the canes. As there were so few insects emerged from these cane sections, the stems, leaves, petioles and tendrils were examined under the microscope for recent emergence holes from both BGSS nymph and parasitoids. A total of 65 emergence holes were counted. The majority of emergence holes were on the apical stems (n = 37) and on tendrils (n = 6, 13, 7, for apical, middle and basal portions, respectively) occurring along the length of the entire canes. Only two emergence holes were counted from leaf petioles and none were counted from middle and basal stems and leaves.

Sentinel Plant Study

To confirm the host association of the emerged parasitoids with the BGSS, three sweet-basil, a chrysanthemum and two wild grape plants were exposed to BGSS lab colonies for 3 days to allow for oviposition. Plants were removed from the colonies and transported to the oviposition survey site to allow for parasitization of BGSS eggs. After three days, the plants were brought back from field, cleaned of any insects and placed into separate cages. Plants were observed daily for any emerging insects. A combined total of 197 BGSS and *Polynema* sp. emerged from the five sentinel plants. Of these, 55 were BGSS nymphs and 142 were *Polynema* sp. (54 males, 88 females). Parasitism rates of BGSS eggs by *Polynema* sp. ranged from 33% on the mum to 78% and 86% on wild grape and basil, respectively.

BGSS and Parasitoid Activity

A total of 12 yellow sticky card traps (11 x 15 cm), were placed at the 2003 oviposition survey site to monitor BGSS adult and parasitoid flight activity. Traps were set up on 9 January 2004 and collected at bi-weekly intervals. Peak trap catch of BGSS adults occurred over the two week period of 11 June to 25 June 2004. Additionally, as soon as wild grape had sprouted and was available for collection, starting on 16 April 2004, twelve 30 cm cane sections were collected at the same bi-weekly sampling intervals. Tendrils were severed from the cane and placed into individual Petri dishes while stems were placed into dual 50 dram vials (25 cm of cane above water to allow for emergence). Plant material was checked daily for emergences of nymphs and parasitoids. Peak emergence of BGSS nymphs and parasitoids was spread over a four week period from 24 July to 20 August 2004. Data compilation is still in progress, however some of the results are shown below in Figure 4.

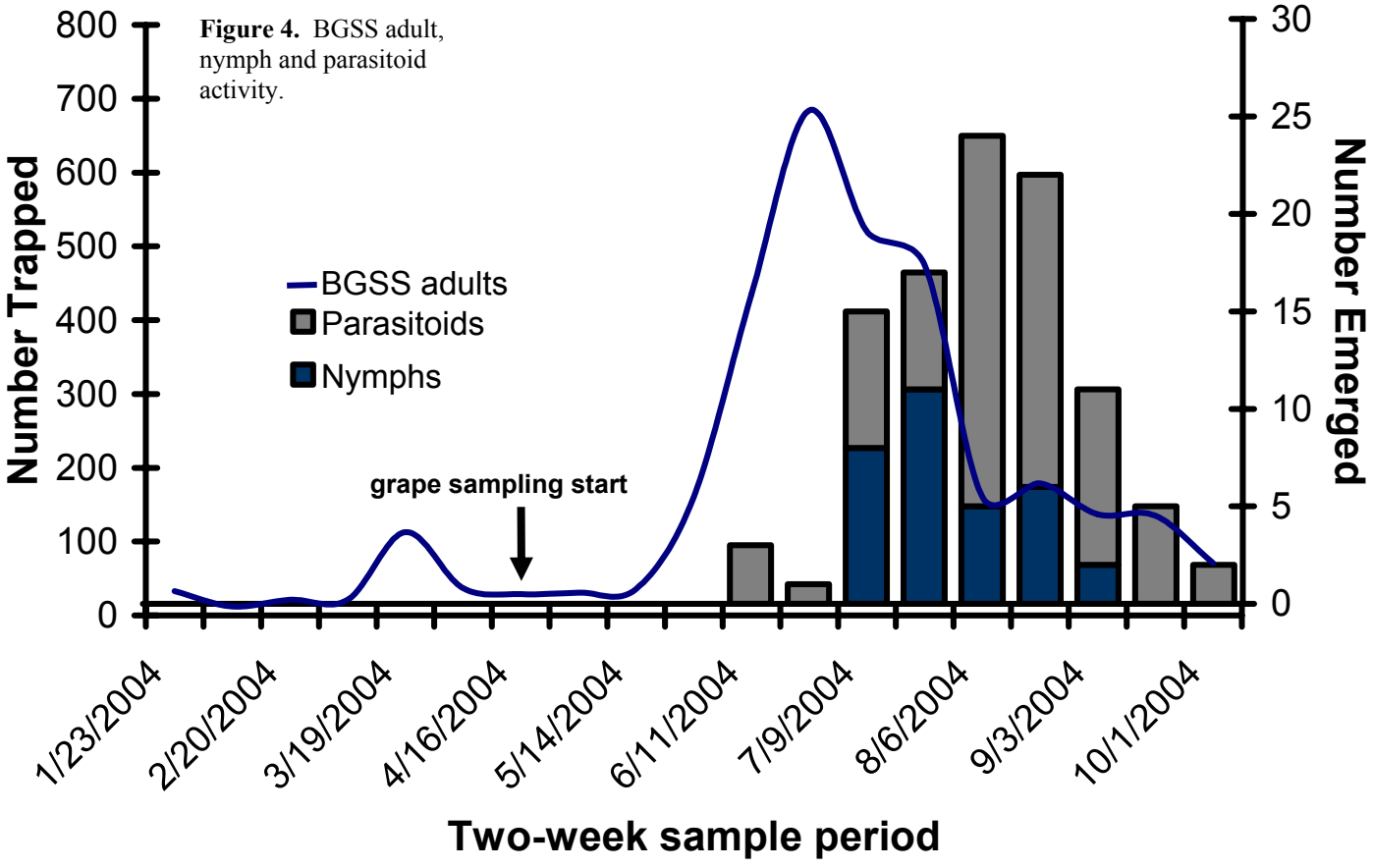

Figure 4. BGSS adult, nymph and parasitoid activity.

***Host specificity testing**:* No-choice tests were conducted with *G. ashmeadi* and STSS eggs. Single, one day old, mated, fed *G. ashmeadi* were exposed to STSS (n = 40 egg masses) and control (GWSS, n = 7 egg masses) eggs on chrysanthemum leaves in individual 100 x 15 mm Petri dishes. Each wasp was supplied one egg mass less than 48 hours of age and allowed 24 hr to parasitize the eggs before removal from the dish. The number of eggs per egg mass ranged from 2-14 ($\overline{x}$ = 5.65) for STSS and 2-19 ($\overline{x}$ = 5.89) for GWSS. Percent parasitism of egg masses ranged from 0-100% for both STSS ($\overline{x}$ = 84.58%) and GWSS ($\overline{x}$ = 71.43%) and was not found to be significantly different (Figure 5, Student's t-test, alpha = 0.05, *P* = 0.37702).

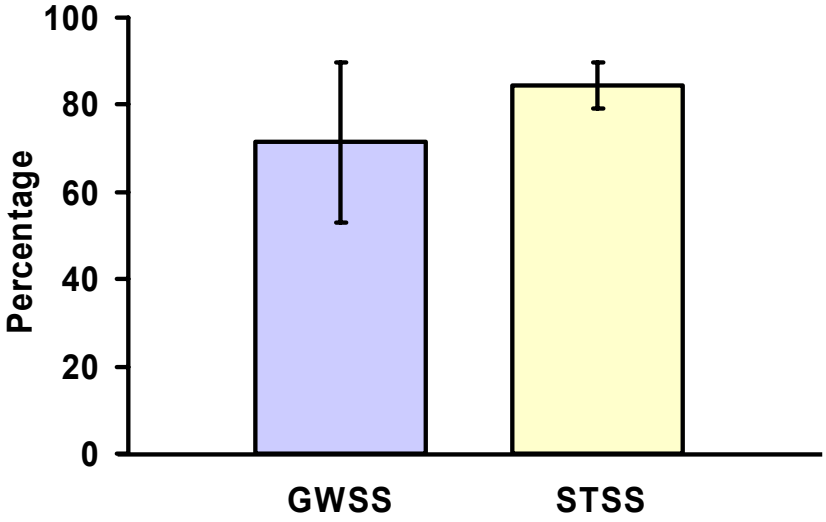

Figure 5: Percent parasitism of STSS and GWSS eggs by *G. ashmeadi* in Petri dish no-choice studies.

CONCLUSIONS

Clearly we now know BGSS oviposition preference on wild grape is for new growth, consisting primarily of the terminal 25 cm of succulent stems and tendrils that occur along the entire length of the grape cane. Additionally we have confirmed two new natural enemy host associations for the BGSS, *G. latipennis* and *Polynema* sp. While these studies were conducted on wild grape, the information acquired may have implications in developing a more complete IPM program involving this native pest species and its associated natural enemies. Overall, the new knowledge of BGSS oviposition preference provides essential information for conducting future non-target effect studies involving the exotic GWSS egg-parasitoids which we have started to investigate. Peak BGSS adult activity measured through trap catches occurred from mid-June to early August while peak emergence of nymphs and parasitoids was spread over a four week period from 24 July to 20 August 2004. Another peak of adult activity may be expected in October once the nymphs have matured into adults. No-choice tests with *G. ashmeadi* and the STSS yielded no significant differences in percent parasitism as compared with GWSS control. It is likely there will be non-target impacts by *G. ashmeadi* in STSS habitats where this parasitoid is able to successfully infiltrate and compete with other resident natural enemies such as *Ufens* and *Zagella* sp. (both Trichogrammatidae)

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FUNDING AGENCIES

Funding for this project was provided by the University of California Agriculture and Natural Resources.

IS THE GLASSY-WINGED SHARPSHOOTER PARASITOID *GONATOCERUS MORRILLI* ONE SPECIES OR A COMPLEX OF CLOSELY RELATED SIBLING SPECIES?

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INTRODUCTION

This is a new proposal that was officially funded in July 2004. This project objective is to determine the status of different *Gonatocerus morrilli* populations. We intend to use three approaches to determine the species identity of different *G. morrilli* populations: (1) Reassessment of key morphological features using scanning electron microscopy to determine if subtle morphological differences exist between *G. morrilli* populations which could possibly indicate species differences (Triapitsyn to conduct this work). (2) Conduct mating compatibility studies to determine if different populations of *G. morrilli* are reproductively isolated, or if mating occurs, whether offspring are viable thereby defining species groups on the basis of successful interbreeding (Hoddle). (3) To determine if molecular differences exist between *G. morrilli* populations collected from different regions by comparing mitochondrial and ribosomal DNA sequences. Molecular dissimilarities of key regions could potentially indicate the existence of different species (Stouthamer). Results from these three areas (morphology, behavior, and molecular) of investigation will be evaluated together to determine whether *G. morrilli* as it is currently viewed is a valid species or whether it is an aggregate of morphologically similar cryptic species.

A classical biological control program is currently underway for glassy-winged sharpshooter (GWSS), which is an exotic pest in California. The native range of GWSS is the southeastern United States and northeastern Mexico (Triapitsyn & Phillips, 2000). GWSS is thought to have invaded California around 1990 as egg masses that were accidentally imported on ornamental plants from Florida. Species of GWSS egg parasitoids not present in California are currently being prospected for in the native range of GWSS. Promising candidate natural enemy species that attack eggs are being imported and released in California for GWSS control (Triapitsyn et al., 1998; Triapitsyn & Hoddle, 2001). Interestingly, one species of egg parasitoid associated naturally with GWSS in California, *Gonatocerus morrilli* (Howard) (Hymenoptera: Mymaridae), is also widely distributed in the home range of GWSS, but at the time of its initial discovery in California, *G. morrilli* had not been intentionally released here and was thought to be native to California. A potential host for *G. morrilli* in California prior to the arrival of GWSS could have been the native *Homalodisca liturata* (Ball) which has had unidentified *Gonatocerus* spp. reared from its egg masses collected in the San Diego area (Powers, 1973). The presence of *G. morrilli* in Riverside in 1980-1984 has been documented (Huber 1988). *Gonatocerus morrilli* is now the second most important natural enemy of GWSS egg masses in California (Al-Wahaibi, 2004).

The success and failure of a number of biological control projects against insect pests and weeds has hinged on the correct taxonomic identification of the target and its natural enemies (Gordh and Beardsley, 1999). Incorrect understanding of the taxonomy and subsequent interrelationships between the target and its natural enemy guild are serious impediments to an efficacious biological control program. For example, *Trichogramma minutum* and *T. platneri* are important commercially available biological control agents that are morphologically indistinguishable but reproductively incompatible (Nagarkatti, 1975). Experimental work and subsequent modeling with these two species of *Trichogramma* has indicated that because pre-mating isolation mechanisms are absent (e.g., pre-mating courtship behaviors that prevent coupling of males and females from different species) severe negative effects on biological control can occur. Negative effects manifest themselves because females that mate with males from different species fail to produce female offspring. This occurs because *Trichogramma* like *Gonatocerus* are haploid-diploid parasitic Hymenoptera. In this haplo-diploid system, fertilized eggs produce female offspring and unfertilized eggs produce male offspring. In situations where incompatible interspecies matings are occurring both species fail to produce females and the potential population growth of both parasitoid species is reduced to levels below the growth rate expected for either species in the absence of the other (Stouthamer et al., 2000).

If different populations of morphologically similar *G. morrilli* from Florida, Louisiana, Texas, and Mexico are indeed valid species that lack pre-mating isolation mechanisms, then the current biological control program against GWSS in California that is attempting to establish these new agents may reduce the current level of control achieved by the precinctive populations of *G. morrilli* in California. This could occur because of male-biased offspring production resulting from incompatible matings across species. The rationale for introducing new strains or races of *G. morrilli* into California is based on the idea that different biotypes of this parasitoid may exist and fill niches not currently occupied by the strain of *G. morrilli* already present in California.

In this grant we propose to determine if geographically distinct populations of *G. morrilli* are part of one continuous interbreeding population or if populations of *G. morrilli* are separate species that can't be easily separated on the basis of

currently employed morphological characters. To do this we intend to combine three separate approaches to determine the species identity of different *G. morrilli* populations: First, we'll reassess key morphological features used to characterize *G. morrilli* with scanning electron microscopy to determine if subtle morphological differences exist between *G. morrilli* populations which could possibly indicate species differences. Such differences - should they exist - may not be easily observed with light microscopy. Second, we'll conduct mating compatibility studies to determine if different populations of *G. morrilli* are reproductively isolated, or if mating occurs, whether offspring are viable thereby defining species groups on the basis of successful interbreeding. Third, we'll determine if molecular differences exist between different *G. morrilli* populations by comparing mitochondrial and ribosomal DNA sequences. Molecular dissimilarities of key regions could potentially indicate the existence of different species, and at the same time allow their identification. Results from these three areas (morphology, behavior, and molecular avenues) of investigation will be evaluated together to determine whether *G. morrilli* as it is currently viewed is a valid species or whether it is an aggregate of morphologically indistinguishable cryptic species.

RESULTS

This project has not commenced. The reason for this is that the recruitment of the post-doc has taken some time. We expect the post-doc to be on-line in early December 2004. We will be formally requesting a no-cost extension for this project.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

SPATIAL POPULATION DYNAMICS AND OVERWINTERING BIOLOGY OF THE GLASSY-WINGED SHARPSHOOTER IN CALIFORNIA'S SAN JOAQUIN VALLEY

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ABSTRACT

The purpose of this project is to define specific environmental constraints that influence glassy-winged sharpshooter (GWSS) population dynamics and overwintering success. We are beginning experiments to determine the temperature-dependent feeding biology of GWSS in temperature-controlled chambers. Experiments are underway in the recently established GWSS Experimental Laboratory on the campus of California State University, Fresno. Adult GWSS feeding and survival under different combinations of host plant type and temperature regimes will be monitored to determine the temperature thresholds for adult feeding activity. Complementary experiments measuring honeydew excretion rates have begun to determine the amounts of excreta collected upon exposed surface(s) of water-sensitive paper and will be compared among different temperature and exposure regimes. Electro-penetration feeding monitoring assays are underway at different temperatures on individually tethered and feeding GWSS adults. Time course examinations of waveforms reveal the frequency and duration of insect feeding behavior under varying environmental conditions. The seasonal population dynamics of GWSS will be monitored on selected host plants placed in different micro-climatic areas of the San Joaquin Valley. Results from these experiments will be coupled with climatological data to help to spatially define where GWSS can be expected to persist in the agricultural landscape and identify where continued management efforts should be directed to limit introductions into currently non-infested areas.

INTRODUCTION

The bacteria *Xylella fastidiosa* (*Xf*) causes economically important diseases of several agronomic, horticultural, and landscape ornamental crops (Pearson and Goheen 1988). The bacterium is transmitted by xylem feeding sharpshooters (Cicadellidae) and spittlebugs (Cercopidae) (Adlerz and Hopkins 1979, Purcell and Frazier 1988). In California, Pierce's disease incidence has been exacerbated following the introduction, establishment and continued spread of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, which is an effective vector of *Xf*. GWSS was first detected in southern California in the early 1990's and populations have since become established in many locations throughout southern portions of the state. First detected in Kern County in 1998, GWSS is now present in the San Joaquin Valley. However, the rapid population expansion first observed in southern California appears to be constrained to discrete regions within agricultural areas of the San Joaquin Valley and incipient, localized populations in urban areas of Fresno, Sacramento, Chico, and San Jose. The continued spread of GWSS into other California localities will almost certainly threaten the economic viability of grapes and other crop species susceptible to infection by various *Xf* strains.

Climate appears to play a significant role in the geographic distribution of diseases caused by *Xf* strains in California and throughout the southeastern U.S. (Purcell 1977, 1980, 1997). Similarly, populations of GWSS in the southeastern US appear to be constrained by climatic factors that limit the pest's establishment and persistence (Pollard and Kaloostian 1961, Hoddle 2004). Presently, limited information exists on the overwintering biology and ecology of GWSS in the San Joaquin Valley of California. An emerging hypothesis is that GWSS may be limited by certain temperature thresholds at, or below, which feeding may be discontinued. In turn, we are designing experiments to carefully determine the thresholds below which feeding discontinues. Additionally, we will determine the critical duration of time spent in this non-feeding state, which may result in increased mortality. The results of the outlined experiments will advance our ability to define the specific environmental constraints that influence GWSS population dynamics and overwintering success. This information will by

increase our present understanding of the overwintering requirements of GWSS with a focus on critical environmental and host species factors that may limit population distribution in the Central Valley of California.

OBJECTIVES

1. Identify the critical environmental constraints that influence the spatial population dynamics and overwintering success of GWSS in California's Central Valley.
2. Characterize the impact of host plant species succession on the overwintering survivorship of GWSS populations that constrain the insect's ability to become established and persist throughout the San Joaquin Valley.

RESULTS

Objective 1

Experiments designed to define the temperature-dependent feeding biology of GWSS are underway at the GWSS Experimental Laboratory on the campus of California State University Fresno (CSUF). Colonies of adult GWSS are maintained at this newly established USDA-ARS research facility in cooperation with research personnel from CSUF, the University of California (Riverside, Berkeley), and the California Department of Food and Agriculture. Plans are to characterize adult GWSS feeding and survival in climate-controlled growth chambers to determine the temperature threshold for adult feeding activity under different combinations of host type and temperature regimes. Adult insects from the rearing colonies, as well as field collected insects in reproductive diapause, will be caged on selected plant species at varying temperatures for different exposure periods in environmental chambers. At the completion of the exposure period(s), the three infested treatments of each plant species will be removed from the chamber and adult GWSS performance and survivorship monitored through the remainder of the adult insect life on the respective test plants in individual screen cages.

In preliminary trials designed to indirectly measure feeding rates, water sensitive paper placed under caged adult GWSS on cowpea collected varying levels of excreta at temperatures of 15.6, 10.0, and 4.6°C (Figure 1). Water sensitive paper strips (2" X 3"), which collect excreted honeydew, are placed adjacent to the plant stem and immediately below a 2" diameter cylindrical Lexan® cage in which adult GWSS are confined on a test plant. In future experiments, the paper will be notched and fit to the plant stem and will be manually replaced on a 4 hour interval over 24 hour intervals. Over the 24 h observations, 12 honeydew clocks will be used for each variety at each of 3 start times corresponding to 0600, 1400, and 2200 h to determine any influence of time of day (Padgham and Woodhead 1988). The amount of excreta collected upon the exposed surface(s) of water-sensitive paper will be compared among different, replicated temperature and exposure regimes to better refine the environmental conditions in which GWSS feeding is restricted or discontinued.

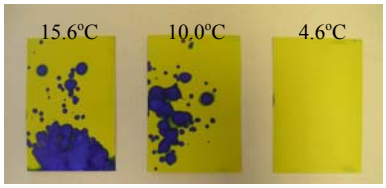

Figure 1. Adult GWSS honeydew collected on water-sensitive paper at varying temperatures over a 24 h interval.

A third set of laboratory experiments are underway using an electro-penetration feeding (EPG) monitoring apparatus to perform waveform analysis at different temperatures. Ten day old adult female GWSS are used in these EPG experiments and are initially placed in separate acclimation cages for 2 hours at the appropriate temperature upon which they will be tested. Preliminary results illustrate differences in the frequency and duration of probing events (green-shaded boxes) of adult GWSS held at temperatures of 15.6, 10.0, and 4.6°C for 12 hour testing intervals on cowpea test plants (Figure 2). Waveform excerpts were taken approximately 225 seconds after the recording began and compressed 2000 times to represent 6.5 hours of recording. These preliminary results indicate that temperature grossly affects GWSS probing behavior between 4.4-15.5 °C. In planned experiments, a total of 5 tethered insects will be simultaneously monitored as experimental replicates at temperatures of 12.2, 10.0, 8.9, and 6.7 °C for exposure intervals of 6, 12, and 24 hour periods. Time course examination of waveforms will reveal the frequency and duration of insect feeding behavior and will help to accurately define the temperature threshold at which ingestion and other waveforms are halted (Serrano et al. 2000).

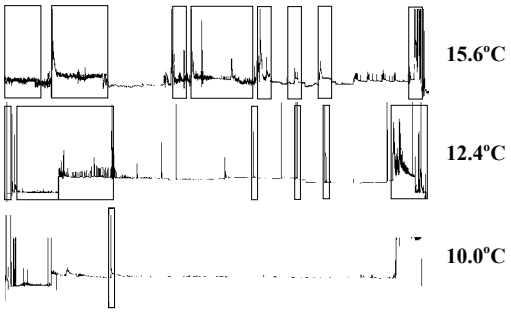

Figure 2. Waveform excerpts (ca. 6.5 h) from a preliminary experimental replicates illustrating the effects of temperature on GWSS probing behavior.

Objective 2

Seasonal population dynamics of GWSS will be monitored on selected host plants placed in different micro-climatic areas of the San Joaquin Valley: 1) the citrus-growing, foothill region of Tulare County; and 2) a GWSS-infested region of the valley floor just west of Porterville in Tulare County. In these experiments, we will examine GWSS survivorship in caged experiments on a selected host plant species. In each cage, fifty second generation GWSS adults, nearing reproductive diapause in the fall season, will be collected from natural infestations and released onto caged plants in late summer. Insects

will be introduced onto potted plants placed in cages and populations monitored monthly throughout the winter period and in the subsequent spring. At each location, four caged replicates of host plant species including the plant species navel orange, grape, and peach will be evaluated individually and in combination. A detailed record of adult GWSS feeding and resting preference will be observed twice monthly throughout the 20 week duration of the experiment beginning November and lasting through March.

CONCLUSIONS

We believe that this recently funded project has a high probability of success both in terms of generating significant new information regarding the overwintering population dynamics of GWSS in California and in providing practical guidance towards management of this pathosystem. This information will further be useful in accurately identifying specific regions of the Central Valley where GWSS overwintering survivorship is greatest and a significant threat of reinfestation is posed. Our research will expand on previous work that has characterized the role of climatic factors in the distribution of *Xf* diseases by defining the specific environmental constraints that influence GWSS population dynamics. Moreover, results from these experiments will be coupled with climatological data in an effort to spatially define those locations where GWSS populations may be unable to successfully overwinter or conversely where populations may find overwintering refuges from extended periods of temperatures that limit adult feeding (Figure 3). Combined with our findings in laboratory bioassays, high resolution (i.e., 1 km scale) raster-based data can be queried to generate predictive maps revealing areas within the Central Valley that may function as "thermal islands", which could favorably support GWSS overwintering populations compared to adjacent agricultural landscapes. As an example, Figure 3 illustrates results of a raster file generated from data collected in January 1993 portraying the number of occurrences where daily maximum temperatures never exceeded 10°C (50°F) for periods of 48 and 96 hours, respectively. With an improved understanding of the climatological limits of GWSS overwintering survivorship, these data can help to spatially define where GWSS can be expected to persist in the agricultural landscape and identify where continued management efforts should be directed to limit introductions into currently non-infested areas. The proposed research will generate critical new information about GWSS spatial population dynamics, thereby contributing towards the development of long-term, economically, and environmentally sustainable management solutions that will directly benefit agricultural producers, crop consultants, and other stakeholders.

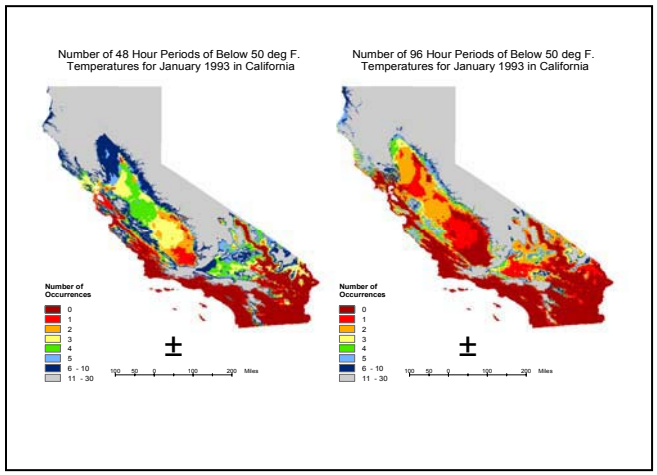

Figure 3. Extended intervals of cool temperatures (< 50°F) January 1993 illustrating microclimatic differences in the San Joaquin Valley

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BIOLOGY AND MORPHOMETRIC ANALYSIS OF GLASSY-WINGED SHARPSHOOTERS REARED ON COWPEA[i]

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ABSTRACT
Stage specific survival, growth, developmental biology, and morphometric analysis of individual glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), were studied in the laboratory at 27 ± 1 ºC, 65 ± 5 RH and 14:10 L:D photoperiod regime, on excised cowpea leaves and stems. Embryonic development of eggs was completed in 7.1 days with 92.6% of the eggs incubated being fertile. The total nymphal period for females (61 $\pm$ 3.0 days) was significantly longer than that of males (53 $\pm$ 1.5 days). Significant differences were observed between the duration of the 5 nymphal stages, with the 2nd being the shortest and the last (5th) the longest for both sexes. Stage specific mortality was similar between instars, ≈ 36.4% of the nymphs reached adult stage, and adult sex ratio was not different from a 1:1 ratio. Based on a cohort of 15 pairs, analysis of life table parameters indicated that populations of *H. coagulata* increased at a rate of 1.045 per day and doubled within 15.6 days. Biometric data comprising body length, head capsule width and hind tibia length were recorded on a total of 276 individuals. The different growth stages were well described by the three biometric parameters. However, analysis of frequency distribution showed that head capsule width was the most suitable parameter for distinguishing the immature developmental stages of GWSS.

INTRODUCTION
The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), is a highly polyphagous xylem-feeder that is indigenous to the southern United States, from Florida to Texas, and northerneastern Mexico (Turner and Pollard 1959). Other than being a minor nuisance in urban environments, the glassy-winged sharpshooter itself causes relatively little direct economic damage or plant loss except for the cosmetic damage to citrus fruits from egg masses deposited into fruits when populations of *H. coagulata* are high (Hix et al. 2003). The most destructive characteristic of GWSS lies in its ability to transmit a plant bacterial pathogen, *Xylella fastidiosa*, one of the causal agents of Pierce's disease (PD) (Redak et al. 2004). However, the recent invasion and establishment of *H. coagulata* in California has dramatically changed the ecology of *X. fastidiosa* and the epidemiology of Pierce's disease (Almeida and Purcell 2003).

Despite the importance and vector status of GWSS, few studies have evaluated its reproductive biology. Little is known about its life table statistics, as published biological studies have not covered the entire life cycle of GWSS. The reasons of the paucity of knowledge on the reproductive biology of GWSS might be the lack of artificial diet-based rearing method for GWSS, as well as the different nutrient requirements of nymphs and adult (Brodbeck et al. 1996).

The present study is focused on developing a simple rearing method for following the development of individual GWSS from egg to adult emergence. We also recorded the longevity and fecundity of adults, and determined the life table statistics of GWSS. Life tables and fertility tables are powerful tools for analyzing and understanding the impact that an external factor has on growth, survival, reproduction, and rate of increase of an insect population (Bellows et al. 1992). As the GWSS undergoes five ecdyses during its development (Turner and Pollard 1959, Brodbeck et al. 1999), it is of significant importance to develop reliable morphological criteria for distinguishing the various nymphal stages.

OBJECTIVES
1. Develop a simple method for rearing individual GWSS from egg to adult on cowpea.
2. Determine the survivorship, egg to adult development time, and reproduction potential of GWSS on cowpea.
3. Examine the growth pattern of this sharpshooter based on three selected biometric parameters that could be used to distinguish the different developmental stages.

RESULTS AND CONCLUSIONS
Biology and Life Table Statistics
The ultimate survivorship of *H. coagulata* on cowpea was 36.4% (Figure 1). The duration of the five instars ranged from 6 to 24 d and was significantly affected by nymphal stage, sex and the sex by developmental stage interaction (Table 1). Within each sex group, the first three instars had the shortest development time, while the last instar (5th) took the longest time to complete for females only (Table 1). The mean total nymphal period of *H. coagulata* on cowpea was 8 d longer for females (61 d) than males (53 d) (Table 2). Out of the 32 *H. coagulata* adults that emerged, 18 were females but the sex ratio was not different from a 1:1 ratio.

Adult longevity was comparable for males (47 d) and females (52 d). For both males and females, no mortality occurred until 20 d after adult emergence. There was a 5 d pre-oviposition period (3 - 9 d) and a 3 d post-oviposition period (0 - 7 d).

A high proportion of females (88%) deposited eggs, with a mean total of 194 eggs per female. The eggs were deposited in clusters under the epidermis layer of cowpea leaves and were mostly in even numbers (93%). Most of the eggs incubated (92.6%) were fertile, and took from 5 to 8 d, with a mean value of 7.1 d, to emerge at 27 ºC.

Life table statistics of GWSS on cowpea are presented on Table 2. Populations of GWSS could multiply at a rate of 33.6 times per generation on cowpea, thus doubling in 15.6 d. Analysis of natality pattern of GWSS revealed that the number of offspring per female was independent of female age, suggesting that food availability might determine the fecundity potential of females.

The successful completion of GWSS life cycle on cowpea suggests that the xylem fluid of this plant has a nutrient profile suitable for both immature and adult stages. The rearing approach used here is quite sample and allowed us to follow each individual GWSS during its development.

Biometric analysis
Values of the three biometric parameters, BDL, HTL, and HCW, varied significantly with the developmental stage (Table 1). Only the grouping for the HCW did not overlap between nymphal stages as indicated by the mean comparison and the distribution of frequency analysis (Table 1, Figure 2). Thus, the HCW could be used as a reliable parameter for distinguishing the five nymphal stages of GWSS.

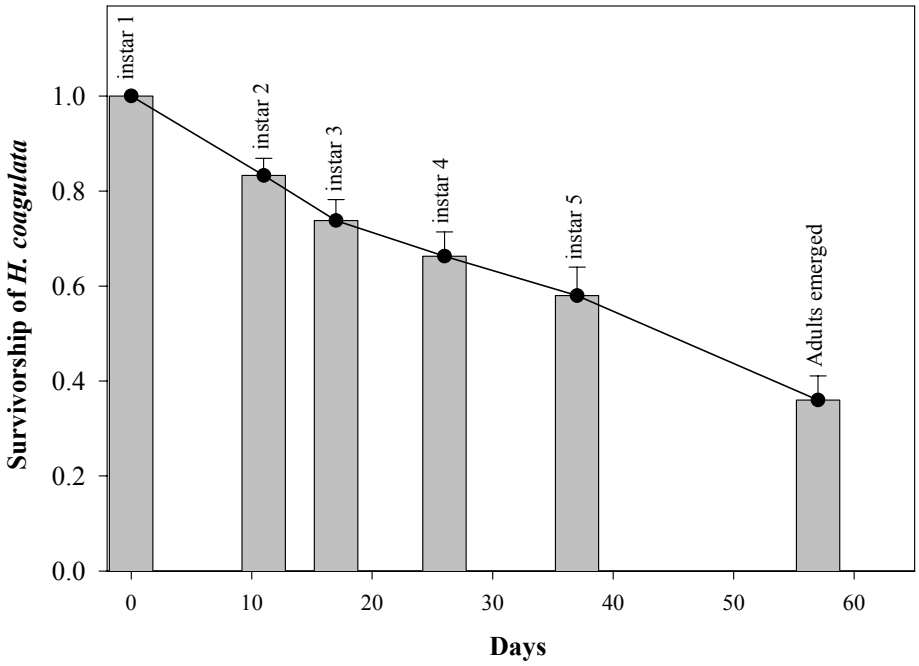

Figure 1. Survival of *H. coagulata* nymphal stages on excised cowpea leaves maintained at 27 °C.

Table 1. Mean[a] developmental duration and size of three biometric parameters of immature stages of GWSS reared on excised cowpea leaves.

Instar	Immature duration ± SE (days)			Biometric parameter ± SE (mm)			
	N	Female	Male	*N*	HCW	BDL	HTL
1	90	10.8 ± 0.9 a BC	10.1 ± 0.9 a BC	76	0.63 ± 0.004 g	2.30 ± 0.03 f	0.86 ± 0.01 g
2	76	6.1 ± 0.5 a C	5.8 ± 0.8 a C	21	0.82 ± 0.015 f	2.98 ± 0.0.09 f	1.19 ± 0.02 f
3	57	8.2 ± 0.9 a BC	8.9 ± 1.2 a BC	27	1.33 ± 0.034 e	5.61 ± 0.24 e	2.03 ± 0.08 e
4	40	12.1 ± 0.7 a B	12.9 ± 0.9a AB	27	1.87 ± 0.013 d	8.63 ± 0.14 d	3.01 ± 0.04 d
5	32	23.7 ± 2.5 a A	14.6 ± 1.8 b A	40	2.13 ± 0.031 c	9.58 ± 0.15 cd	3.19 ± 0.04 d
Total	32	60.9 ± 2.9 a	53.0 ± 1.5b	-	-	-	-

[a] Means followed by the same small case letter within each row and by the same capital letter within each column are not significantly different (P > 0.05), Ryan-Einot-Gabriel-Welsch multiple range test (REGWQ). N, represents the sample size.

Table 2. Fecundity and life table parameters of GWSS reared on excised cowpea leaves.

Parameter	*n*	*Fecundity**	r_m	R_o	*G*	*DT*	λ
Mean	15	193.7	0.044	33.6	79.3	15.6	1.045
95% LCI		154.2	0.040	22.38	74.7	14.1	1.041
95% UCI		233.2	0.049	44.75	83.8	17.0	1.050

* Mean fecundity of gravid females only, i.e., 13 females; *n*, number of pairs included in analysis; r_m, jackknife estimate of the intrinsic rate of increase; R_o, net reproductive rate; *G*, mean generation time (in days); *DT*, population doubling time (in days); and λ, finite rate of increase; LCI = lower confidence limits and UCI = upper confidence limit

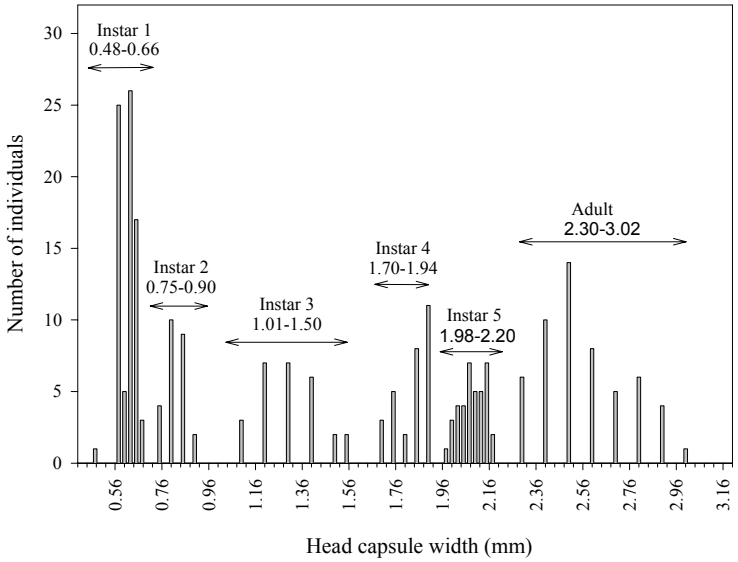

Figure 2. Distribution of head capsule widths of GWSS nymphs and adults.

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EFFECTS OF USING CONSTANT AND CYCLICAL STEPWISE-INCREASING TEMPERATURES ON PARASITIZED AND UNPARASITZED EGGS OF THE GLASSY-WINGED SHARPSHOOTER DURING COLD STORAGE

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Reporting Period: The results reported here are from work conducted from December 1, 2003 to October 1, 2004.

ABSTRACT
Glassy-winged Sharpshooter (GWSS) egg masses, deposited on *Euonymus japonica* cuttings, were stored 1d after oviposition at either a constant temperature of 12ºC or under a regime that cycled daily, stepwise, (10, 11, 12, 13ºC @ 6h intervals) under an 8L:16D photoperiod. After storage under the cycled temperature regime for 15 and 20d, the hatch was 74 and 63%, respectively. Control hatch at 20d was about 80% and 50% after storage at a constant 12ºC. The survival to adulthood, length of the nymphal stage, and the fecundity of the adult females were all affected by cold storage during the egg stage, regardless whether the temperature was held constant or cycled. Survival to adulthood was reduced 30 to 40% and the time required to complete the nymphal stages was significantly longer than the control. The number of eggs oviposited by females and length of the ovipositional period after being held at 12ºC during the egg stage was about one-half that of the control group, while the values for the 20d cycled group are yet to be determined. The rates of parasitism and emergence by *Gonatocerus ashmeadi* decreased with the length of time that 1-d-old unparasitized GWSS eggs were stored under the cycled regime. When held up to 25d in storage, parasitism by wasps and emergence of their progeny remained statistically similar. After 50d of storage, parasitism and progeny emergence dropped 30% and 20%, respectively. After a storage period of 25d, parasitoid emergence from parasitized eggs stored at a constant 4.5ºC was significantly higher than those stored similarly at 4ºC. The cycled stepwise-increasing temperature regime of 4.5, 6.0, and 7.5ºC changing at 8h intervals yielded a significantly higher parasitoid emergence than a cycled regime of 4, 6, and 8ºC. When stored under the regime starting at 4.5ºC, for 10, 20 and 25d, the emergence of wasps was 66%, 59% and 59%, respectively. Parasitized eggs stored under this regime for 80d produced no wasps.

INTRODUCTION
Studies on cold storage of insects and their eggs have shown that developmental age, storage temperature, time in storage, and inherent species tolerance are the factors which influence survival after a cold storage period (Leopold 1998). The most effective temperature for storage of GWSS eggs was determined to be 12ºC (Leopold et al. 2003). Storage of 1-d-old GWSS eggs at 10ºC resulted in no survival after only 8d period. Storage at 13 and 14ºC resulted in high survival and parasitism by *Gonatocerus ashmeadi* and *G. triguttatus* at 20d, but in-storage hatching of the GWSS eggs occurs after 30d and successful parasitism by the wasps decreases under these constant temperature regimes. The within-host cold tolerance of the *Gonatocerus spp*. is significantly greater than that of the unparasitized GWSS eggs. Emergence of the wasps occurs at temperatures $\geq$ 5ºC when the parasitized eggs are stored < 20d. Since certain conditions, such as temperature variation and fluctuation and high or low humidities have been reported to enhance survival of insects and their parasites during cold storage (Iacob and Iacob 1972, Gautum 1986, Liu and Tian 1987, Leopold et al. 1998), the present study was initiated to determine whether we could lengthen the survival time of GWSS eggs and the egg parasitoid by varying the temperature while in storage. We were especially interested in determining whether any latent damaging effects of chilling would be expressed, beyond diminished emergence, that might affect the quality of previously cold-stored insects.

OBJECTIVES
1. Compare the cold tolerance of GWSS eggs stored at a constant temperature with eggs stored under a cycled stepwise temperature regime and evaluate the post storage developmental time of nymphs and reproduction of adults.
2. Compare the effects of cold storage of unparasitized GWSS eggs under constant and cycled stepwise low temperatures regimes on the subsequent parasitism and emergence of *G. ashmeadi*.
3. Determine whether a cycled stepwise cold temperature regime enhances the shelf-life of parasitoids while in host eggs.

RESULTS AND CONCLUSIONS
Cold storage of Unparasitized GWSS Eggs
GWSS egg masses deposited on *Euonymus* cuttings were stored in incubators set at constant (12ºC) and cycling stepwise-increasing temperatures (10, 11, 12, and 13 ºC @ 6h intervals) under an 8L:16D photoperiod for varying lengths of time. After removal from storage, the cuttings bearing GWSS egg masses were incubated at room temperature (ca. 22 ºC) to record egg hatch. After storage at 12ºC for 30d, 52.7 ± 10.2% of 1-d-old eggs (n = 102), 50.7 ± 7.1% of 3-d-old eggs (n = 87) and 44.7 ± 5.1% of 5-d-old eggs (n =61) hatched. However, no hatching was observed after 30d storage. When stored at the stepwise cycling temperature (10-13 ºC) for 15, 20, and 25d, the hatch of 1-d-old eggs was 73.9 ± 11.1% (n = 142),

62.6 ± 9.1% (n = 98) and 44.6 ± 9.1% (n = 104), respectively. There was a significant difference in percentage hatch of 1-d-old GWSS eggs between the control eggs (83.0 ± 7.4%, n = 317) and those eggs stored for 25d (F = 3.939, df = 3,45, P = 0.014), but no significant differences were found between those groups stored in the cold for 15 or 20 days and the control. After storage for 80d under the daily cycled regime, no hatching was observed.

To determine effect of cold storage during GWSS egg stage on nymphal development and adult reproduction, newly hatched nymphs from eggs stored at 12 ºC for 20 days, and at the daily cycled temperature regime for 15 and 20 days were reared on sunflower plants until they emerged as adults. When the characteristic patch of brochosomes was observed on the forewings of the adult females (brochosomes were considered as the sign that females had mated), they were then individually maintained on sunflower plants and their egg mass output recorded until death occurred. Our preliminary data (Table 1) shows that 50% of nymphs from eggs stored at 12ºC for 20 d and 50% and 40% of nymphs held under the stepwise temperature regime for 15 days and 20 days, respectively, successfully developed into adults. In comparison with the control groups, GWSS males and females from those eggs that had been exposed to either cold storage regime took significantly longer to complete their nymphal stages (Table 1). There were no differences in male and female developmental times among the nymphs that hatched from GWSS eggs that had undergone cold storage. The number of eggs produced/female and the ovipositional period was considerably greater for the control groups and approached 2-fold differences.

Effects of Cold Storage of GWSS Eggs on Parasitism and Emergence by G. ashmeadi

Following storage in incubators set at a constant 12 or 12.5ºC and also at the stepwise cycled regime as described above, GWSS egg masses were exposed to caged *G. ashmeadi* colonies for 2 days at room temperature (ca. 22 ºC) and under an 10L:14D photoperiod. Before statistical analysis, the data recorded for parasitism and emergence were square-root transformed to correct non-normality because the number of eggs/mass was not constant.

After storage at 12ºC for 30d, 69.6 ± 11.7% of the 3-d-old GWSS eggs (n = 90) and 47.7 ± 11.7% (n = 106) of the 1-d-old eggs were successfully parasitized by *G. ashmeadi*. The percentage wasp emergence was 68.5 ± 11.3 for 3 day-old eggs and 35.3 ± 10.0% for the 1 day-old eggs. There were no significant differences in the incidence of parasitism, as determined by egg dissection, ($F = 4.034$, $df = 1,14$, $P = 0.066$) and emergence ($F = 1.728$, $df = 1,14$, $P = 0.211$). Further, *G. ashmeadi* successfully parasitized about 77% of the 4-d-old , 52% of the 5-d-old, and 45% of the 3-d-old GWSS eggs stored at 12.5ºC for 30d, and 46% of 3-d-old eggs stored for 50d. As above, there were no significant differences between parasitism and emergence in any of the comparable groups (data not shown).

When stored under the cycled stepwise temperature regime (10-13 ºC), the parasitism ($F = 14.934$, $df = 8,137$, $P < 0.001$) and emergence ($F = 13.661$, $df = 8,137$, $P < 0.001$) of 1-d-old GWSS eggs by *G. ashmeadi* varied significantly with storage time (Table 2). More than 75% of GWSS eggs stored up to 25d were successfully parasitized and there was no significant difference in the incidence of parasitism between the control (92.1 ± 9.9%, n = 172) and the eggs stored for 15, 20 or 25d ($F = 1.764$, $df = 3,35$, $P = 0.172$). However, percentage emergence for the eggs stored for 25d was significantly lower than that for the control (91.7 ± 2.7%, n =172) ($F = 3.250$, $df = 3,35$, $P < 0.033$). Further, there were no significant differences in percentage emergence between the control eggs and the eggs stored for 15 or 20d ($P = 0.099$). After storage for 65d, < 44% eggs were parasitized by *G. ashmeadi*, and about 23% of wasps emerged, which was significantly lower than for eggs for stored for 25d or less. When stored for over 80d, the percentage parasitism and emergence were less than 12% and 7%, respectively. When these data were analyzed via a regression analysis, the percentage parasitism and emergence vs. storage time was found to be inversely correlated (Figures 1 and 2).

Cold Storage of GWSS Eggs Parasitized by G. ashmeadi

The experimental conditions for this study consisted of a constant 4 or 4.5ºC storage temperature and 2 daily cycled stepwise-increasing regimes (4, 6, and 8ºC or 4.5, 6, and 7.5ºC - each temp. changing at 8h intervals) under an 8 L: 16 D photoperiod. After the parasitized eggs were stored at 4 ºC for 10d, only 7.2 ± 5.0% (n = 85) of the wasps emerged, which was significantly lower than those parasitoids similarly stored at 4.5ºC (33.5 ± 7.2%, n = 280), 20 days (33.9 ± 6.9%, n = 114) or 25 days (21.7 ± 5.2%, n = 125) ($F = 11.962$, $df = 4,66$, $P < 0.001$). No parasitoids (n = 164) emerged from host eggs stored at 4ºC for 20d (Figure 3). When parasitoids were stored within hosts under the cycled stepwise temperature regime starting at 4 ºC, percentage emergence was 42% (n =126) at 10 d, 8 % (n = 420) at 20d and 0% (n = 184) at 25d (Figure 4). However, for parasitized eggs stored at the other cycled regime starting at 4.5ºC, the wasp emergence was at or above 60% throughout the 25d of storage. Thus, the percentage emergence for the parasitoids stored under the stepwise regime starting at 4.5ºC for 10-25d was significantly higher than that for the eggs stored for 15d under the regime starting at 4ºC ($F = 48.237$, $df = 5, 114$, $P < 0.0001$). Parasitoids within GWSS eggs did not emerge after storage for 80 days, but further research is needed to ascertain if maintenance of the *Euonymus* cuttings that bear the egg masses during the storage period is causing a problem.

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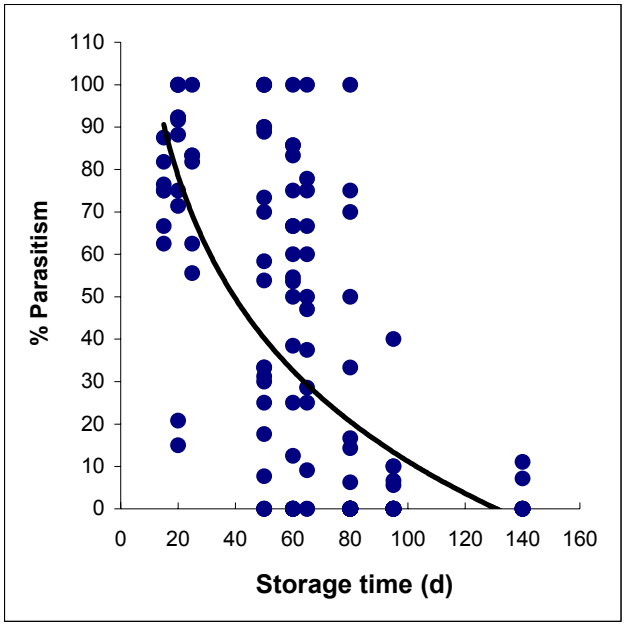

Figure 1. Relationship of the % parasitism (y) of *G. ashmeadi* to storage time (x) of the GWSS eggs at stepwise temperatures (10~13ºC)(y = 5.10 + 1393.18/x, $r = 0.58$) ($F = 68.24$, $df = 136$, $P < 0.001$)

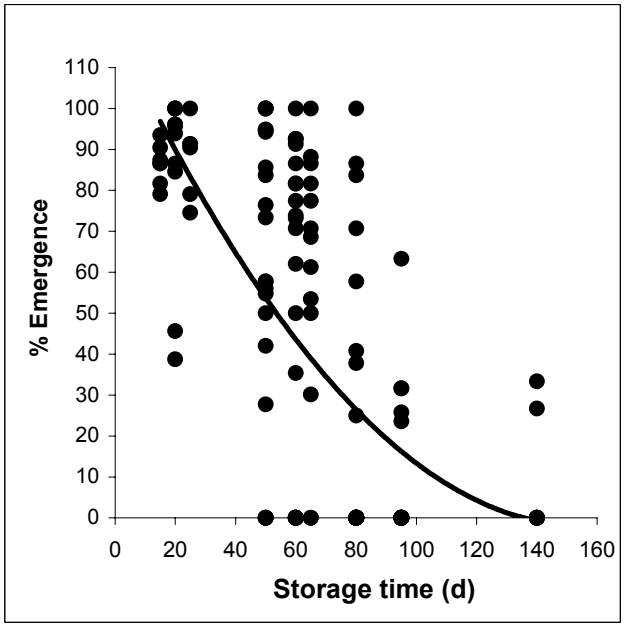

Figure 2. Relationship of the % emergence (y) of *G. ashmeadi* to storage time (x) of the GWSS eggs at stepwise temperatures (10~13ºC) (y = -0.35 + 1286.50/x, $r = 0.59$) ($F = 79.01$, $df = 136$, $P < 0.001$).

Table 1. Egg hatch, development time of nymphs and reproduction of adults for GWSS eggs stored under different temperature conditions (mean ± SE).

Storage conditions	Egg hatch (%)	Development time of nymphal stage			Adult reproduction	
		% survival	Male (d)	Female (d)	No. eggs/ female	Ovipositional period (d)
Control (25ºC)	82.9 ± 7.4	80.2	35.9 ± 0.5 a	35.3 ± 0.6 a	1068.8 ± 187.7	113.4 ± 49.6
12ºC for 20 d	52.7 ±10.2	50.0*	43.9 ± 0.9 b	42.5 ± 0.7 b	589.3 ± 81.9	65.7 ± 26.0
10-13ºC for 15 d	73.9 ± 5.4	50.0*	43.0 ± 0.7 b	41.0 ± 1.2 b	662.7 ± 111.1	65.0 ± 11.2
10-13ºC for 20 d	62.6 ±10.3	40.0*	43.0 ± 3.5 b	41.9 ± 0.4 b	In progress	In progress

Only 1 replicate. Means within a column followed by different letters were significantly different at the significant level of 0.05 (SAS Proc GLM with LSD). Data for egg hatch were square-root transformed before analysis.

Table 2. Parasitism and emergence by *G. ashmeadi* on the GWSS eggs exposed to the daily stepwise temperature regime (10, 11, 12, 13ºC - changing at 6h intervals) for 15 to 140 d.

Storage time	No. egg masses	No. eggs	Parasitism (mean % ±SE)	Emergence (mean % ± SE)
15 d	7	88	74.99 ± 3.20 a	68.25 ± 3.11 a
20 d	11	106	76.98 ± 9.26 a	67.18 ± 9.23 ab
25 d	6	69	77.76 ± 6.58 a	57.15 ±13.49 ab
50 d	21	226	47.75 ± 8.15 b	41.89 ± 8.05 bc
60 d	23	208	37.27 ± 7.49 b	28.69 ± 6.61 c
65 d	13	126	44.36 ± 8.69 b	22.58 ± 7.11 c
80 d	31	253	11.79 ± 4.68 c	7.31 ± 3.84 d
95 d	17	193	4.25 ± 2.40 c	1.90 ± 0.89 d
140 d	9	96	2.02 ± 1.38 c	2.02 ± 1.38 d
			$F = 14.934$	$F = 13.661$
			$df = 8,137$	$df = 8,137$
			$P < 0.001$	$P < 0.001$

Means within a column followed by different letters were significantly different at the significant level of 0.05 (SAS Proc GLM with LSD). Data were square-root transformed before analysis.

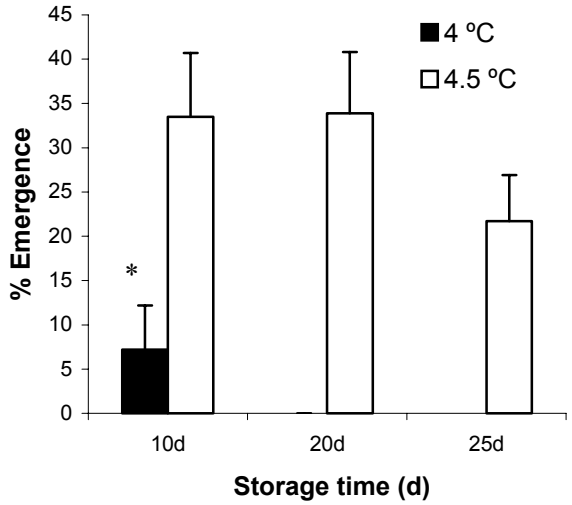

Figure 3. Percentage emergence of *G. ashmeadi* from GWSS eggs stored at constant temperatures for 10-25 d. Bar marked by an asterisk represents a significant difference ($P < 0.05$).

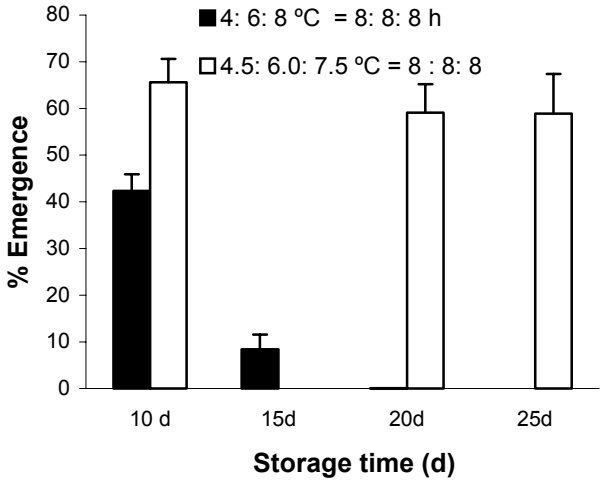

Figure 4. Percentage emergence of *G. ashmeadi* from the GWSS eggs stored at stepwise temperatures for 10-25 d. Bar marked by an asterisk represents a significant difference ($P < 0.05$).

PARASITISM OF THE GLASSY-WINGED SHARPSHOOTER: FUNCTIONAL RESPONSES AND SUPER-PARASITISM BY THE EGG PARASITOID *GONATOCERUS ASHMEADI.*

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Reporting Period: The results reported here are from work conducted from December 1, 2003 to October 1, 2004.

ABSTRACT
The functional responses and super-parasitism by the egg parasitoid, *Gonatocerus ashmeadi*, on *Homalodisca coagulata* eggs were related to host age and density when studied under laboratory conditions. Parasitism of Glassy-winged Sharpshooter (GWSS) eggs, 1-, 3-, 5-, 7- and 9-d-old, was measured at 22 ± 1ºC and under 10L:14D regime. For each host age, 10-60 eggs were exposed to an individual parasitoid for 24 h. The functional responses for the parasitoids to host eggs of all age groups most closely fit the type II and III models of Hollings (1959) and Hassell (1978) which relate to the elapsed time for accomplishing the behavioral events associated with parasitism of the host as modified by host density. The instantaneous attack rate by parasitoids on 1-d-old host eggs, as specified in the type III model, was significantly greater from that of the other ages. This rate was also greater in the type II model but was not statistically significant. The total number of host eggs parasitized varied significantly with host density and age of the eggs, but not when analyzed by a host x density interaction. Host age and density, as well as the host x density interaction, contributed significantly to the differences found in length of development time of *G. ashmeadi* within host eggs. The wasps exhibited a tendency towards super-parasitism at relatively high parasitoid-to-host ratios. The maximum number of parasitoid eggs found in a single host egg was 18. The development time and eclosion of the parasitoids had no correlation with parasitoid-to-host ratios. Frequencies of super-parasitism for *G. ashmeadi* displayed an aggregated distribution over all observed host densities.

INTRODUCTION
The effectiveness of parasitoids in regulation of a pest population is highly dependent on their ability to search for and handle hosts in a varying ecosystem. This effectiveness has been traditionally related to the functional response of a parasite or predator (Hassell 1978, Fujii *et al.* 1986). The functional response is defined as the relationship between the numbers of prey taken by the predator as a function of prey density (Holling 1959). The functional response is an essential component of the dynamics of host-parasitoid relationship, and is an important determinant of the stability of the system (Oaten and Murdoch 1975). Functional response analyses are commonly used to help predict the potential for parasitoids to regulate host population (Solomon 1949, Oaten and Murdoch 1975). Successful parasitoids have the ability to discriminate among parasitized eggs, avoid super-parasitism and minimize the waste of time and energy associated with their searching and parasitizing behaviors (Godfray 1994). However, under certain circumstances, superparasitism might be adaptive (van Alphen & Visser 1990). Further, when mass-rearing solitary parasitoids for use in an augmentative release program, super-parasitism represents a waste of the production colony's potential output. This report presents the progress on investigations determining certain aspects of the functional responses and super-parasitism by the parasitoid, *G. ashmeadi*.

OBJECTIVES
1. Investigate the response of *G. ashmeadi* to GWSS eggs of different ages and determine the effects of host egg age on functional response parameters and parasitism.
2. Determine effect of host densities and ages with respect to developmental time of wasps.
3. Investigate relationship between super-parasitism by the wasp at different host densities and effect of super-parasitism on wasp emergence and development time.

RESULTS
Functional Responses
There was a significant increase in the numbers of *H. coagulata* eggs of different ages parasitized by egg parasitoid, *G. ashmeadi,* with an increase in host density (Table 1). At the host densities of 40, 50, and 60, the numbers of eggs parasitized were significantly higher than that of relatively low densities of 10 and 20 over all host ages. The number of 1-d-old eggs parasitized was slightly greater than that of 5-, 7- and 9-d–old-eggs. A two-way ANOVA, with age and density as factors, revealed that the number of eggs parasitized varied significantly with host age ($F = 3.64$, $df = 4,299$, $P = 0.007$) as well as host densities ($F = 88.43$, $df = 5,299$, $P < 0.0001$). There was no significant effect of age × density interaction on the number of host eggs parasitized ($F = 0.44$, $df = 20, 299$, $P = 0.899$).

The functional responses of *G. ashmeadi* parasitizing host eggs at the various ages showed that the shape of the functional response curves were affected by differences in the parasitization rates of *G. ashmeadi*. At all host ages, the *G. ashmeadi* functional response data most closely fit the type II and III models. Coefficients of determination (r^2 values) for type II and III curves were very similar (Table 2). The instantaneous attack rates (a) and handling time (T_h) estimated

from type II functional response models varied slightly but were not significantly different among host ages (Table 3). The *a* value for 1-d-old eggs was slightly higher than that for other ages when data were fit to a type II functional response model. The estimate for handling time (time spent on eggs) by the wasps for all host egg ages did not vary significantly. When the data were fitted to a type III functional response model, the *a* value estimated for 1-d-old eggs was significantly higher than that for host eggs of 3-, 5-, 7- and 9-d-old. However, the handling time of *G. ashmeadi* for all egg ages was similar, ranging from the value of 0.032 to that of 0.040.

Effect of Host age on Parasitoid Development Time

The development time of *G. ashmeadi* within host eggs varied significantly with host density and host age (Table 4). Within the 1-, 3-, 5-, 7- and 9-d-old host eggs, the mean development time (± SE) of the parasitoid was 16.0 ±1.0 d (n = 1435), 18.9 ± 1.8 d (n = 996), 18.3 ± 1.5 d (n = 1181), 17.6 ± 1.2 d (n = 961) and 17.8 ± 1.5 d (n = 1254), respectively. The parasitoid within 1-d-old sharpshooter eggs developed significantly faster than that within other ages ($F = 766.41$, $df = 5, 5826$, $P < 0.0001$). A two way ANOVA further showed that host age ($F = 999.47$, $df = 4, 5826$, $P < 0.0001$) and density ($F = 58.26$, $df = 5, 5826$, $P < 0.0001$) contributed significantly to the development time of *G.ashmeadi*. The significant interactive effect on development time occurred between host age and density ($F = 62.82$, $df = 20, 5826$, $P < 0.0001$).

Super-parasitism.

Maximum number of parasitoid eggs in one host egg was 18. The level of super-parasitism of *G. ashmeadi* (Table 5) varied significantly with increasing host density ($F = 225.17$, $df = 5,549$, $P < 0.0001$). The mean number of parasitoid eggs per sharpshooter egg at 1:1 parasitoid-to-host ratio is significantly greater than that at other ratios. When the parasitoid-to-host ratio increased to > 1:15, host eggs pooled from each host density were almost all parasitized. There was a significant positive correlation between the number of parasitoid eggs per host egg and parasitoid-to-host ratio ($F = 1231.69$, $df = 548$, $r = 0.8319$, $r^2 = 0.692$, $P < 0.0001$). *G. ashmeadi* is a solitary parasitoid and normally only one wasp emerges from each egg of its host. In treatments with high host densities such as at 1:1 and 1:5 parasitoid ratios, the percentage of parasitoid eclosion was significantly higher than in low-density treatments ($F = 3.996$, $df = 4,243$, $P = 0.004$)(Table 5). However, there is no correlation between parasitoid-to-host ratio and percentage of parasitoid eclosion ($F = 3.29$, $df = 242$, $r = 0.1140$, $r^2 = 0.013$, $P = 0.071$). Although there was a significant statistical difference in development time of the parasitoid within the host egg among different parasitoid-to-host ratios ($F = 46.851$, $df = 4, 1862$, $P < 0.0001$), the maximum difference was only about 0.7d.

For *G. ashmeadi*, x^2 goodness-of-fit analyses of parasitoid egg numbers per host egg revealed that frequencies of super-parasitism were significantly different from the expected Poison distribution over all host densities ($x^2 = 231.291$, $df = 4$, $P < 0.0001$). The relationship between the variances (S^2) and means (m) was described by Taylor's power law (Taylor 1961) as: $\log S^2 = -0.4384 + 1.0288 \log m$ ($r^2 = 0.604$, $df = 28$, $F = 42.78$, $P < 0.0001$, where $b = 1.0288 > 1$, indicating an aggregated distribution of super-parasitism for *G. ashmeandi* over all experimental parasitoid-to-host ratios.

CONCLUSIONS

The studies on the functional responses of *G. ashmeadi* to GWSS eggs of different ages and densities in the laboratory have improved our understanding of the interactions between the parasitoid and host egg. Because this parasitoid fits the II and III functional response models in relation to different host ages, it further confirms that the wasp has the capacity of effectively parasitizing eggs throughout most of the embryonic development of the GWSS. Further, studies on super-parasitism of *G. ashmeadi* provide valuable information for the mass-rearing and field release of this parasitoid. Our results indicate that super-parasitism occurs when the parasitoid-to-host ratio is greater than 1:15. Super-parasitism results in a waste of the reproductive potential of this species because *G. ashmeadi* is a solitary-developing wasp and usually only one parasitoid emerges from one GWSS egg.

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Table 1. Parasitism by *G.ashmeadi* on *H. coagulata* eggs of different ages at varying densities.

	Mean No. Parasitized (SE)				
Density	**1d**	**3d**	**5d**	**7d**	**9d**
10	9.5(1.3) a	8.7(2.2) a	8.9(1.6) a	9.0(2.2) a	9.1(1.1) a
20	18.1(1.6) b	15.5(3.2) b	14.8(3.4) b	14.6(3.9) ab	14.7(3.3) ab
30	22.9(3.0) c	17.9(8.4) b	22.0(5.8) c	19.8(7.7) bc	18.7(4.1) b
40	26.5(4.7) cd	22.2(9.8) bc	25.1(7.3) cd	22.7(5.6) c	25.8(6.2) c
50	30.3(7.5) d	25.6(10.0) cd	29.4(5.1) de	23.9(11.9) cd	29.5(13.1) c
60	34.8(4.7) e	30.7(6.9) d	32.2(4.5) e	29.9(7.3) d	30.1(3.4) c
	$F = 43.12$	$F = 11.02$	$F = 31.69$	$F = 10.59$	$F = 16.96$
	df = 5,59	df = 5,59	df = 5,59	df = 5,59	df = 5,59
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.001$

Means in a column followed by different letters are significantly different ($P < 0.05$, GLM) in ANOVA (Duncan).

Table 2. Coefficients of determination for functional response regression models of *G. ashmeadi* to *H. coagulata* eggs of different ages.

Age of Eggs (d) [a]	**Type I (r^2)**	**Type II (r^2)**	**Type III (r^2)**
1	0.7776	0.9729	0.9727
3	0.4979	0.8993	0.8992
5	0.7260	0.9607	0.9608
7	0.4783	0.9038	0.9036
9	0.5872	0.9280	0.9280

[a] *G. ashmeadi* targeted host densities ranged from 10 to 60 sharpshooter eggs per experimental container. Type I functional response model was evaluated using SAS PROC GLM whereas Type II and III models were evaluated using SAS PROC NILN to generate r^2 values indicating best fit.

Table 3. Type II and III functional response parameters of *G. ashmeadi* when parasitizing *H. coagulata* eggs of different ages.

Functional response model	**Host age (d)**	**Instantaneous attack rate (a ± SE)[a]**	**Handling time (T_h ± SE)[a]**
Type II	1	0.5782 ± 0.0626 a	0.0300 ± 0.0004 a
	3	0.4544 ± 0.0959 a	0.0315 ± 0.0105 a
	5	0.5013 ± 0.0640 a	0.0286 ± 0.0058 a
	7	0.5064 ± 0.1088 a	0.0377 ± 0.0099 a
	9	0.4831 ± 0.0849 a	0.0296 ± 0.0082 a
Type III	1	2.8131 ± 2.2011 a	0.0342 ± 0.0056 a
	3	1.0137 ± 0.5410 b	0.0333 ± 0.0117 a
	5	1.4394 ± 0.6301 b	0.0316 ± 0.0067 a
	7	1.3858 ± 0.9508 b	0.0403 ± 0.0113 a
	9	1.2495 ± 0.6620 b	0.0322 ± 0.0094 a

[a] Instantaneous attack rate (a) and handling time (T_h) estimated by SAS PROC NLIN and pairwise compared among host ages using indicator variable (0 or 1) for age.

Table 4. Development time of *G. ashmeadi* within *H. coagulata* eggs of different ages when parasitized at varying densities.

	Development time (SE) at age:				
Density	**1d**	**3d**	**5d**	**7d**	**9d**
10	15.9(0.6) d	21.0(2.1) a	17.9(1.6) c	15.7(1.4) e	17.6(1.4) c
20	16.5(0.8) a	18.5(1.6) c	18.0(1.1) c	18.3(0.9) a	18.3(0.8) b
30	16.5(0.7) b	18.6(2.2) c	18.8(1.3) b	18.1(0.9) ab	19.1(1.2) a
40	16.1(0.8) c	18.3(2.2) c	17.8(1.5) c	18.0(1.0) bc	16.9(1.6) d
50	16.0(1.4) cd	18.6(1.5) c	19.5(1.2) a	17.8(0.6) c	17.4(1.4) c
60	15.5(0.7) e	19.4(1.0) b	17.4(1.0) d	17.2(1.2) d	18.1(1.2) b
	$F = 45.39$	$F = 37.00$	$F = 88.13$	$F = 84.08$	$F = 73.93$
	df = 5,1434	df = 5,995	df = 5,1180	df = 5,960	df = 5,1253
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Means in a column followed by different letters are significantly different ($P < 0.05$, GLM) in ANOVA (Duncan).

Table 5. Number (mean ±SE) of G. ashmeadi eggs per host egg, percentage of emergence and development time at different parasitoid-to-host egg ratios.

Parasitoid-	**No. parasitoid / host**		**% Emergence**		**Development time**	
host ratio	**N_1**	**Mean ±SE**	**N_2**	**Mean ±SE**	**N_3**	**Mean ±SE**
1:1	50	10.40 ± 4.86 a	NA	NA	NA	NA
1:5	100	3.02 ± 1.69 b	11	97.6 ± 1.7 a	141	18.02 ± 0.07a
1:10	100	2.24 ± 1.16 c	15	98.9 ± 0.6 a	136	18.20 ± 0.06 b
1:15	100	1.66 ± 0.89 d	77	93.6 ± 1.5 b	490	18.30 ± 0.04 b
1:20	100	1.20 ± 0.59 d	70	91.7 ± 1.0 b	263	18.25 ± 0.04 b
1:25	100	1.15 ± 0.58 d	71	90.0 ± 1.7 b	833	18.77 ± 0.03 c

Means in a column followed by different letters are significantly different ($P < 0.05$, GLM) in ANOVA (Duncan). N_1 represents the number of dissected host eggs, N_2 represents the number of egg masses observed, and N_3 is the number of parasitoid emerging from host eggs.

FUNDING AGENCIES
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GLASSY-WINGED SHARPSHOOTER'S POPULATION DYNAMICS AS A TOOL FOR ERADICATING GLASSY-WINGED SHARPSHOOTER POPULATIONS

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ABSTRACT
Our results indicate that **1)** GWSS populations in untreated areas have been declining steadily during the last three years. Current populations are only 10 to 20% as dense as those during 2001-2002. **2)** Forecast analysis indicates that, if the current trend is extrapolated, GWSS populations in untreated areas should decrease to negligible numbers some time after winter, 2008, and before summer 2013, depending on *Citrus* species. However, **3)** analyses of the data sets currently available, show that adult GWSS densities are cycling around a possible equilibrium level of 600 adults in Valencias and 950 adults in lemons, when left untreated. The period encompassed by the data sets for Tangerines and Grapefruit is still too short for this type of analysis. **4)** Overall, less than 30% of the first instar nymphs survive to the fifth instar nymphs, and less than 15% of these nymphs survive to become adults. **5)** During this last winter (2003-2004), overwintering adult densities declined in grapefruit, tangerines, and oranges but they increased in lemons, in the absence of any significant production of nymphs. The latter suggests that adult GWSS were moving among trees and cultivars due to changes in the nutritional and/or moisture status of these trees. We will use the xylem fluid samples currently being analysed, to test this hypothesis.

INTRODUCTION
It is widely recognized that disrupting *Xylella* transmission and preventing Pierce's disease (PD) epidemics requires Glassy-winged sharpshooter (GWSS) population levels to be exceedingly scarce. Recognizing critical points in GWSS' annual population cycles will allow us to identify the spatial and temporal scales during which GWSS populations are vulnerable to control measures timed to coincide with critical densities in its populations that can drive its local populations nearly extinct. In addition, determining whether GWSS populations will continue to decrease and eventually stabilize in the absence of pesticides but in the presence of parasitoids is of the utmost importance. Currently, almost all citrus groves infested with GWSS in California are treated. The groves at Agricultural Operations, University of California Riverside, are an exception. Our work in these untreated groves provides a means of exploring the dynamics of GWSS populations in untreated citrus groves exposed to egg parasitism. The results from these studies might also suggest the expected dynamics of GWSS populations inhabiting urban environments where GWSS is under little or no control except by egg parasitoids.

Our results to date suggest that GWSS has a major reproductive period during the spring and a second reproductive period during autumn. This autumn generation involves a dense egg population laid by the GWSS arising from the spring generation but very few of these eggs mature to become adult GWSS. Furthermore, nymphal mortalities are quite high, only about 30% of the first instar nymphs reach the last nymphal stage, and less than 15% of these first instar nymphs survive to become adults, but this varies between Citrus varieties. Although the source of this egg and nymphal loss still needs to be explored, we have measured egg parasitism ranging from 78% to 92% during the second half of the year. It is at this point that the GWSS may be vulnerable to a selective control measures. Our studies also showed an 80 to 90% decline during the last three years in valencias and lemons. The period of one year during which we have been sampling tangerines and Grapefruit is still too short to conduct a worthwhile analysis for these varieties (See figures 1 to 4). Next year's samples from the four citrus varieties will be crucial in testing whether the pattern in GWSS' dynamics continues or is transient.

OBJECTIVES
This project seeks to characterize GWSS' spatial and temporal dynamics involved in its annual population cycles on its dominant host, i.e. *Citrus sp.* We seek to identify periods in this cycle during which selective control measures, appropriately

timed might drive the GWSS population below its critical density, thus leading to its local extinction. To fulfill this goal, we propose the following objectives:

1- Expand our current studies to follow GWSS population dynamics at a landscape level, including urban areas, using our whole host plant sampling technique.
2- Determine the relative contribution of the principal host plants to the adult GWSS production in each generation.
3- Determine whether correlations exist between GWSS' population dynamics on a given host tree and the host's xylem chemistry and whether this correlation explains GWSS' variable performance seasonally on different host plants.
4- Use this information to identify critical periods during GWSS' annual population cycle where selective control strategies might drive its local populations nearly extinct.

RESULTS

The number of adult GWSS in untreated valencia and lemon trees at the Agricultural Operations fields, University of California, Riverside has declined during the two and a half years of our study (Figure 1 through 4). GWSS densities on Tangerines and Grapefruit trees involves one and a half GWSS generations and, thus, is too short a period for a meaningful analysis of GWSS on these citrus varieties. Figures 1 and 2 show the mean number of adult GWSS obtained from three valencias and three lemons per sampling date, during the two and a half year sampling period. It is clear that a significant downward trend has occurred in the number of GWSS adults during the two and a half years. Peak densities have decreased by 67% for Valencias and 75% for lemons between 2002 and 2003. At the time of this report, we had not reached the peak adult densities for 2004, which typically occur in late August to early September. The GWSS samples from Tangerines and Grapefruit also show a decreasing trend. The average number of new adults produced in the three Valencia and the three lemon trees per sampling date also declined during the two and a half year study (Figure 1 & 2).

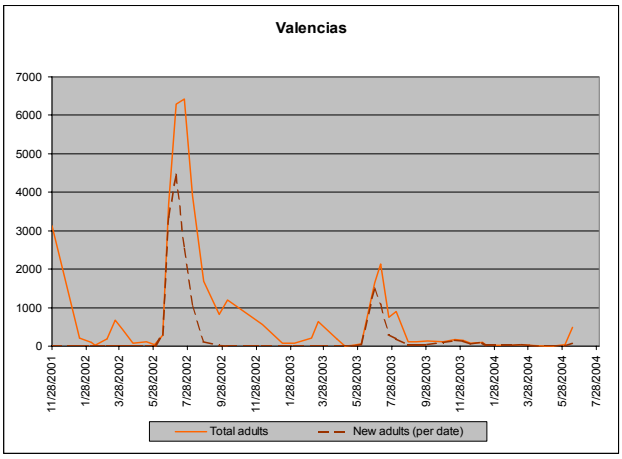

Figure 1. Actual adult GWSS densities (solid line) and newly produced adults per date (dotted line) in an untreated Valencia grove.

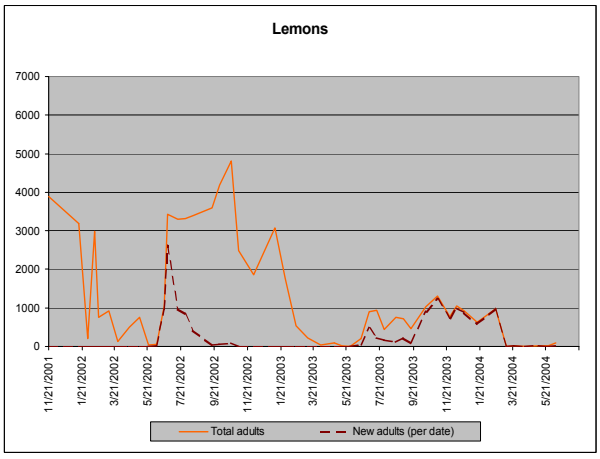

Figure 2. Actual adult GWSS densities (solid) and newly produced adults per date (dotted) in an untreated Lemon grove.

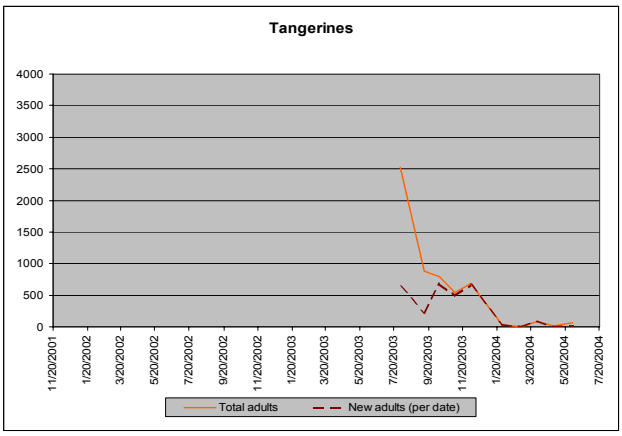

Figure 3. Actual adult GWSS density since Fall 2003 in an untreated Tangerine grove.

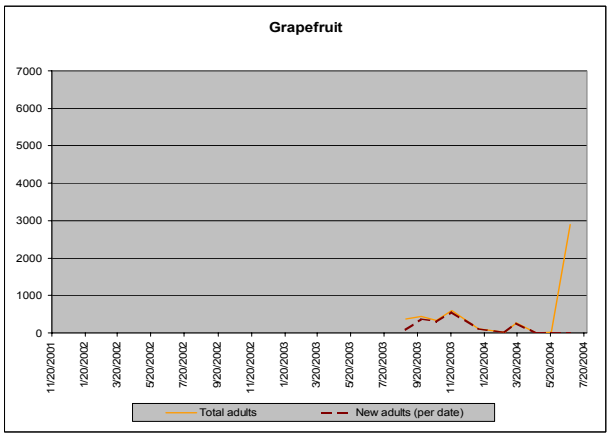

Figure 4. Actual adult GWSS density since Fall 2003 in an untreated Grapefruit grove.

A more interesting analysis using the population samples from Valencia and Lemon trees is presented in Figures 5 and 6. We plotted the total adult and the newly emerged (red-veined) adult density using a logarithmic scale. We then used a forecasting technique on these data for Valencia and Lemons separately, i.e. the lines in Figures 5 and 6 which show what would happen if the current trend is extrapolated until it reaches zero. Although it is unlikely that GWSS will ever reach zero, we use these plots to estimate a minimum and a maximum date when we expect these populations to reach their minimum. These two dates are estimated by the lines crossing the X-axis in each graph and encompass the time period during which we estimate that GWSS adult populations will reach their minimum.

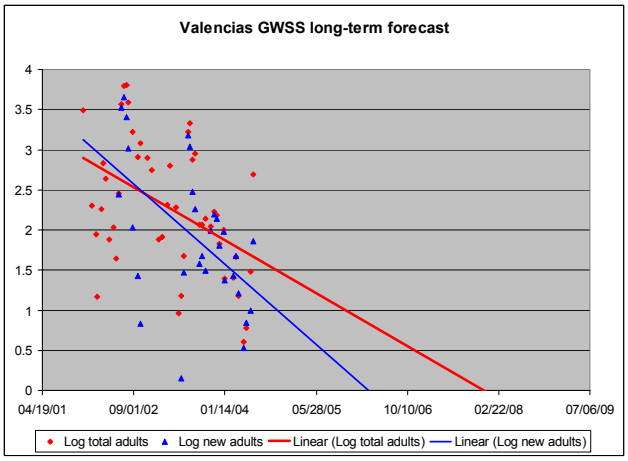

Figure 5. Logarithm of total and new adults in Valencias with trend lines showing expected "zero density" dates.

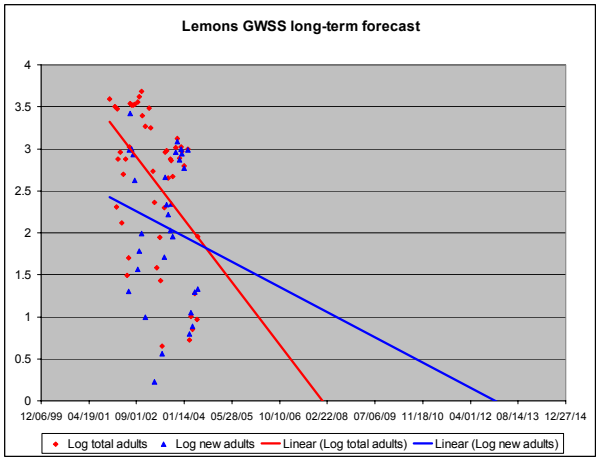

Figure 6. Logarithm of total and new adults in lemon with trend lines showing expected "zero density" dates.

If the current trend continues for several years the adult GWSS will reach their minimum densities within the next three to six years. However, as new data are collected and plotted on these graphs a more refined minimum density will be obtained but it is extremely unlikely that the GWSS densities will become extinct. A second and even more powerful technique can be used to analyze the GWSS dynamics (figures 7 and 8). These figures need some explanation. What they show is a plot of GWSS adult densities at any a specific date, as a function of the density at a previous time interval. In our case, it is the density of adult GWSS at a given week, as a function of the density two weeks previously. In a sense, it explores the effect on a given date's density, of the density two weeks prior. When plotted in this manner, we get a phase diagram that shows whether the GWSS population density is cycling and, if it is cycling, it shows the density around which the population is likely to be cycling. Figure 7 shows the phase diagram for Valencias. The point, at which the two diagonal lines cross, shows the density around which adult GWSS population cycles, generation after generation. This does not mean that the population will reach an equilibrium density at exactly that density. Rather, it indicates the density around which the population will cycle. For Valencias, this equilibrium density is about 600 adults per tree, and for lemons, it is about 950 adults per tree. Thus, this analysis suggests that GWSS will never reach "zero density," but will alternatively reach densities above and below the cycling density at different times of the year and in different years. The data sets for tangerines and grapefruit do not encompass a sufficient enough period of time to allow this kind of analysis. We will need at least another year of GWSS data before we can conduct this analysis using the forecasting technique. At the same time, a longer dataset for Valencias and lemons will likely improve the accuracy of this analysis.

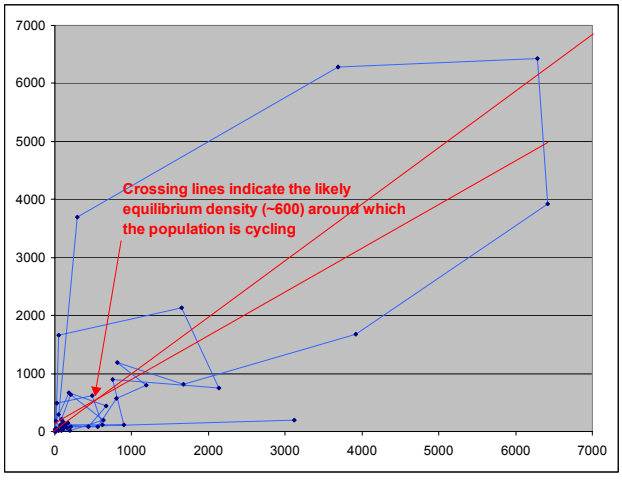

Figure 7. Phase diagram for adult GWSS dynamics in GWSS Valencias (see text).

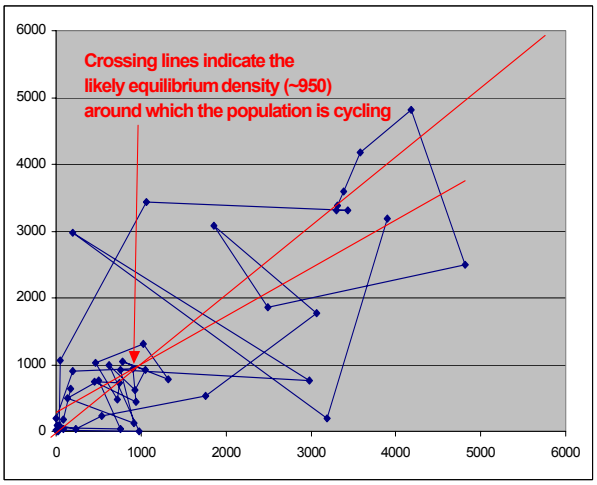

Figure 8. Phase diagram for adult dynamics in Lemons (see text).

CONCLUSIONS

Our work in untreated citrus groves has enabled us to explore what happens to uncontrolled GWSS populations. After an additional year of data, the GWSS densities on valencias and lemons are sufficient to allow us to tentatively forecast the time at which the GWSS will attain their minimum densities on each host cultivar. The analyses show that GWSS are decreasing at a rate that, if sustained, may drive GWSS populations to very low levels. The first technique used predicts minimum densities for GWSS to be achieved during the next three to six years. The second technique, the phase diagram, indicates that an extinction of GWSS is unlikely, and that the populations on valencias and lemons are each cycling around an equilibrium point. During periods when populations are above their equilibrium density, we are likely to see GWSS densities above 1000 adults per tree. In addition, we have shown that GWSS populations manifest different dynamics in different places. As the populations become less dense, their dynamics will bring stability, allowing GWSS to recolonize areas where densities are low when GWSS adults move from areas where GWSS densities remain high (see figure 4, grapefruits as an example). This type of behavior, called metapopulation dynamics at it is known to bring stability in a wide range of biological systems were animals can readily move from one place to another. This appears to be the case for the GWSS and we expect to see these type of dynamics to emerge in the next few years.

FUNDING AGENCIES

Funding for this project was provided by CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

MYCOPATHOGENS AND THEIR EXOTOXINS INFECTING THE GLASSY-WINGED SHARPSHOOTER: SURVEY, EVALUATION, AND STORAGE

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Reporting Period: The results reported here are from work conducted from December 2003-October 2004

ABSTRACT
A species of *Hirsutella,* the primary pathogen of GWSS in the southeastern US, has been the major focus of our research this past year. Due to the fastidious growth requirements of this fungus and the presence of numerous saprobic fungi associated with mycosed GWSS, a major effort has been made to design a series of gene-specific primers to be used to detect these diseases in field collected samples. Molecular-based diagnosis is being used to examine the hundreds of mycosed insects collected during the 2003 and 2004 regional surveys. A second effort has been directed at examining the seasonal incidence of this disease in an experimental crape myrtle plot. A number of parameters such as crape myrtle variety, host density, mist irrigation (humidity) have been found to influence the onset of *Hirsutella* in GWSS populations. Current laboratory research is being directed at examining transmission of the lab culture to both GWSS and to alternate insect hosts. In addition, culture filtrates of all of the fungi collected from GWSS are being assessed for the presence of active metabolites.

INTRODUCTION
We are not aware of any studies that have examined the insect pathogens associated with populations of GWSS. In general, the lack of pathogens (viral, bacterial, or protozoa) in leafhopper populations may be related to their piercing-sucking feeding behavior. In most cases, these pathogen groups are transmitted orally and would likely need to inhabit the xylem tissue to infect leafhoppers. Pathogens that are transmitted *per os* are typically affiliated with insects with chewing mouthparts. Thus, entomopathogenic fungi, which do not need to be ingested in order to infect insects, are considered to contain the primary pathogens of sucking insects. Indeed, the primary pathogens operating against insects such as whiteflies, scales, aphids, spittlebugs, plant hoppers, and leafhoppers are insect fungi (for listing see USDA-ARS Collection of Entomopathogenic Fungal Cultures at http://www.ppru.cornell.edu/mycology/catalogs/catalog). We commonly observe all mobile stages of GWSS exhibiting mycoses in north Florida and we are identifying them and assessing their impact.

OBJECTIVES
1. Identify and archive all the major pathogens affiliated with GWSS populations.
2. Estimate the distribution, frequency and seasonality of the major diseases of GWSS.
3. Screen the pathogens for exotoxins with potential toxicity to GWSS and other arthropods.
4. Confirm infectivity of the isolates and the exotoxins and determine which if any pathogens may serve as microbial controls of GWSS and other leafhopper vectors.

RESULTS
Pathogen Distribution
In the past field season we continued to survey the incidence of disease in GWSS populations in the Southeast. The purpose of this survey was twofold: first, to piece together a better picture of the distribution of the Glassy-winged Sharpshooter in the area. Secondly, it gave us the opportunity to investigate the varieties and incidence of fungal pathogens associated with this host. The survey area encompassed four states, Mississippi, Louisiana, Alabama, and Texas. A series of live GWSS and a total of 95 mummified GWSS were collected from sites in these states. In most cases, the external characters mimicked those observed on the cadavers collected from sites in Georgia, South Carolina, and Florida in 2003. The presence of various opportunistic fungi on field-collected samples has limited our abilities to culture the more fastidious slow growing species of *Hirsutella*, *Sporothrix*, and *Pseudogibellula.* The aforementioned fungi were identified last year to be key entomopathogens isolated from GWSS populations. After multiple cycles of isolation we were able to isolate target fungi from only about 10% of these insects, the vast majority of cultures contained saprobic fungi. In order to confirm the presence of the *Hirsutella* (the primary pathogen) we have developed and optimized PCR primers within unique intron motifs of both the actin and tubulin genes that have been matched with primers from the open-reading frame. Control reactions have demonstrated that these primer combinations are able to specifically amplify the GWSS *Hirsutella* from DNA extracted from mummies. This

technology is being used to screen the more than 250 DNA samples extracted from mycosed GWSS collected from throughout the southeastern US. This work will be summarized and submitted for publication in December 2004.

Analysis of the Dynamics of the Hirsutella in GWSS Populations

A field plot containing 14 cultivars of crape myrtle (total 224 trees) was established at the NFREC. Four subplots, each containing 40 trees, were established within this stand. Two subplots were fitted with an overhead mist irrigation system that was operated 15 minutes every hour, 24 hours a day. Throughout the summer, trees were sampled by counting both the live GWSS and number of mycosed GWSS. Mycosed GWSS were flagged and their positions on the trees were noted. It should be noted that throughout the season the species of *Hirsutella* accounted for virtually 100% of the disease on the GWSS. Preliminary analysis demonstrated a non-uniform distribution of live GWSS and mycosis GWSS in the plot. In part this could be related to both the cultivar and/or to the presence the misting irrigation system. The cultivars attractive to GWSS ('Osage', 'Miami', 'Tonto') contained higher levels of mycosed GWSS. Irrigated crape myrtle, regardless of the cultivar, contained significantly higher mycosed GWSS than did the non-irrigated trees. Currently, the field data from this season is being combined with the positional (cardinal orientation) data and will be subjected to additional statistical analysis

CONCLUSIONS

We have identified and have in culture several isolates of a primary pathogen and potential GWSS biological control agent, *Hirsutella sp*. Molecular methods have been established and are being used to diagnosis GWSS collected from sites throughout the southeastern US. This past field season the dynamics of *Hirsutella* has been examined in replicated crape myrtle plots.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

POPULATION DYNAMICS AND INTERACTIONS BETWEEN THE GLASSY-WINGED SHARPSHOOTER AND ITS HOST PLANTS IN RESPONSE TO CALIFORNIA PHENOLOGY

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INTRODUCTION

The focus of this research is to determine the relative phenology (the timing of biological events as influenced by the environment and intrinsic biological phenomena) of host plant use by glassy-winged sharpshooter (GWSS), other leafhopper vectors and natural enemies, and *Xf* in ornamental, agricultural and CA native host plants in key CA locations in climatically different regions: Coastal (Piru, Ventura County), Inland (Redlands, San Bernadino County), and South (Pauma Valley, San Diego County). As year 1 of a 3 year study, we plan to replicate this years' observations (only if continued CDFA funding is reinstalled and received) using fresh host plants at the same locations, and full analyses of results will not be available until after all data is collected. The findings of this first season are therefore presented as preliminary results.

This research will be used to develop a GWSS performance database on the host plant species that are identified as truly critical to GWSS survival, which is needed to fully support decision making, and to supplement what is observed in the field. Currently, no quantitative data is available on the relative suitability of single or multiple hosts most relevant in Southern California's agriculture, landscape or native vegetation, to GWSS growth and development. This project will provide this baseline information, identify host plant limitations at different life stages and will ultimately identify key nutrients responsible for this phenomenon.

OBJECTIVES

Use 25 different host plant species in 4 replicates per location at three locations: Coastal (Piru, Ventura County), Inland (Redlands, San Bernadino County), and South (Pauma Valley, San Diego County) to:

1. Determine the age structure and utilization of GWSS on the host plants throughout the season
2. Determine the GWSS egg parasitization and mortality, together with the presence of general predators on the host plants throughout the season
3. Determine GWSS fecundity and feeding rate on selected host plants
4. Determine the presence of XF in host plants at three times during the season
5. Determine the chemical composition of the host plant xylem fluids at tree times during the season.

RESULTS

From April onwards, the GWSS age structure and resident generalist predators on 25 different host plants were observed weekly. In four replications, 25 potted (5gal) host plants were used to test the preference of resident GWSS at 3 Southern California locations within unsprayed citrus orchards. For each replication 25 plant pots were placed in a completely randomized block design within the rows. Each block was enclosed in a 5x5ft square pen made with chicken wire. Plants were hand watered 2-3 times per week. The plant species were selected for their common ornamental or agricultural use or their status as orchard weeds or their occurrence in foothill and riparian environments in Southern California (Table 1).

Batch samples from each of the host plant species were tested for the presence of *Xf* on three occasions between April and July. With the exception of one *H. helix* batch sample in May, all batch samples tested negative. In follow-up tests of single *H. helix* plants, no individual plant tested positive for *Xf*.

Table 1 Mean number of egg masses, adults and nymphs recorded per GWSS host plant species in Piru, Redlands and Pauma Valley, California.

Plant	Plant name	Common name	Egg masses[1]	Adults[2]	Nymphs[3]
1	*Hibiscus* sp.	'Mrs. J. E. Hendrey' hibiscus	3.42 ± 1.064 abc	10.50 ± 4.265 a	3.42 ± 0.908 ab
2	*Lagerstroemia indica*	Crape Myrtle	9.58 ± 1.607 de	34.25 ± 20.350 a	17.92 ± 5.113 d
3	*Nerium oleander*	Oleander (white)	O	19.75 ± 8.294 a	10.17 ± 2.925 bc
4	*Gardenia jasminoides*	'Mystery' Gardenia	1.50 ± 0.832 ab	0.42 ± 0.193 a	2.17 ± 0.842 ab
5	*Citrus sp.*	Valencia Orange	2.42 ± 1.314 abc	13.15 ± 3.175 a	11.17 ± 3.164 c
6	*Photinia sp.*	Red Tip Photinia	6.67 ± 2.021 cd	2.08 ± 0.763 a	4.92 ± 1.681 abc
7	*Eucalyptus cinerea*	Silver Dollar Tree	0.50 ± 0.167 a	0.33 ± 0.188 a	0.50 ± 0.289 a
8	*Vitis vinifera*	Thompson Seedless Grape	11.17 ± 2.49 e	14.42 ± 3.019 a	29.75 ± 6.516 e
9	*Euonymus japonica*	Silver Queen	1.92 ± 0.654 ab	0.92 ± 0.358 a	0.25 ± 0.131 a
10	*Ligustrum japonicum*	'Texanum' Wax Leaf Privet	1.58 ± 0.617 ab	1.25 ± 0.494 a	3.25 ± 0.970 ab
11	*Agapanthus africanus*	Lily of the Nile	2.00 ± 0.834 ab	1.08 ± 0.336 a	0.42 ± 0.193 a
12	*Hedera helix*	English ivy	0.33 ± 0.243 a	1.08 ± 0.763 a	0.83 ± 0.297 a
13	*Sonchus oleraceus*	Sowthistle	O	O	0.08 ± 0.083 a
14	*Chenopodium berlandieri*	Lambsquarter	O	0.33 ± 0.188 a	0.33 ± 0.256 a
15	*Malva neglecta*	Cheeseweed	O	O	0.92 ± 0.288 a
16	*Senecio vulgaris*	Common Groundsel	O	O	O
17	*Rhus integrifolia**	Lemonade Berry	0.33 ± 0.263 a	0.58 ± 0.193 a	1.17 ± 0.767 a
18	*Heteromeles arbutifolia**	Toyon	2.00 ± 0.872 ab	0.33 ± 0.188 a	0.67 ± 0.497 a
19	*Baccharis pilularis**	Coyote Brush	1.25 ± 0.740 ab	0.92 ± 0.609 a	1.42 ± 0.434 a
20	*Lonicera subspicata**	Honeysuckle	0.08 ± 0.083 a	0.17 ± 0.112 a	0.08 ± 0.083 a
21	*Opuntia basilaris**	Beavertail Cactus	O	O	0.33 ± 0.333 a
22	*Oenothera speciosa*	Mexican Evening Primrose	0.33 ± 0.067 a	0.25 ± 0.131 a	1.42 ± 0.452 a
23	*Populus candicans*	Cottonwood	4.92 ± 1.493 bc	205.67 ± 96.643 b	54.25 ± 8.927 f
24	*Platanus occidentalis*	"Bloodgood" Sycamore	13.33 ± 3.404 e	12.75 ± 4.961 a	6.58 ± 1.694 abc
25	*Prunus subhirtella*	Akebone Ornamental Cherry	13.83 ± 4.606 e	17.08 ± 8.164 a	4.67 ± 1.689 abc

* California native plant

O life stage not recorded on host plant species

[1] Mean number of egg masses recorded on host plant species over all three locations (different letters indicate significant differences, Kruskal Wallis t=133.69, P<0.0001).

[2] Mean number of adults recorded on host plant species over all three locations (different letters indicate significant differences, Kruskal Wallis t=154.54, P<0.0001).

[3] Mean number of nymphs recorded on host plant species over all three locations (different letters indicate significant differences, Kruskal Wallis t=194.54, P<0.0001).

When considering life stages at the different locations, more egg masses were found on the host plants in Pauma valley between June 24 and August 19 compared to both Piru and Redlands in the same period (unequal variance: Kruskal Wallis: t=7.237, *P*=0.027) (Fig. 1a). The numbers of eggs per egg mass was significantly higher in Pauma (ANOVA df=2, F=10.93, *P*<0.001), a larger portion of the eggs were parasitized in Pauma (ANOVA df = 2, F = 10.67, *P*<0.001), with no difference in emergence of eggs masses (ANOVA df=2, F=3.04, *P*=0.05). The portion survival of eggs per egg mass is lowest in Pauma (ANOVA df=2, F=10.80, *P*<0.001) (Table 2).

Of the parasitized egg masses recorded in Piru, all were *Gonatocerus* sp., but in Redlands 6% were parasitized by *Trichogramma* sp as were 4% of the egg masses from Redlands. T he survival of *Trichogramma* parasitized egg masses was 0.595 ± 0.0544 significantly lower than the survival of *Gonathocerus* parasitized egg masses 0.764 ± 0.011 (unequal variance: Kruskal Wallis t=11.89, *P*=0.000563). No differences were found between the egg mass size and the fraction parasitized for *Trichogramma* or *Gonatocerus* (results not shown).

Table 2 The survival, fraction parasitized and fraction emerged parasitoids recorded in GWSS egg masses in Piru, Redlands and Pauma Valley, California.

	Location			ANOVA		
	Piru	Redlands	Pauma Valley	df	F	*P*
N	197	172	557			
#eggs/egg mass	11.56 ± 0.467 a	12.02 ± 0.499 a	13.81 ± 0.278 b	2	10.93	<0.001
Survival	0.847 ± 0.0237 b	0.795 ± 0.0254 b	0.725 ± 0.0141 a	2	10.80	<0.001
Fraction parasitized	0.666 ± 0.029 b	0.676 ± 0.031 b	0.545 ± 0.017 a	2	10.67	<0.001
Fraction emerged parasitoids	0.804 ± 0.0288 a	0.848 ± 0.0312 a	0.762 ± 0.0187 a	2	3.04	0.051

No egg masses were recorded on oleander, sowthistle, cheeseweed, lambsquarter, common groundsel and beavertail cactus. Over all sites the mean number of egg masses recorded was largest on sycamore, cherry and grape, followed by crape myrtle and photinia (Table 1). The number of egg masses per host plant species differed significantly for crape myrtle, eucalyptus, grape, primrose and cottonwood on which fewer egg masses were found in Piru and Redlands than in Pauma (results not shown). In Piru, most egg masses were recorded on sycamore and cherry, followed by grape. In Redlands, most egg masses were recorded on grape, followed by crape myrtle and photinia, which had more egg masses than sycamore and cherry. In Pauma most eggmasses were recorded on crape myrtle, grape, sycamore and cherry followed by photinia. Because of unequal variances Kruskal Wallis was used for these analyses with P<0.0001 in all cases (results not shown).

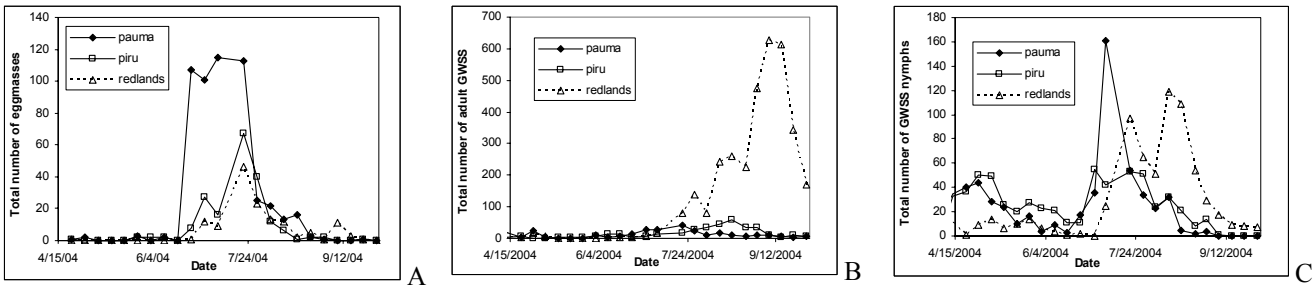

Figure 1: Total number of GWSS egg masses (A), adults (B) and nymphs (C) recorded between April and October 2004, on 100 host plants located in a citrus orchard in Piru, Redlands and Pauma Valley, CA.

When considering GWSS adults at the different locations, more were found on the host plants in Redlands between June 16 and October 1 compared to both Piru and Pauma in the same period (unequal variance: Kruskal Wallis: t=8.4481, *P*=0.0146) (Fig. 1b). Adults were not recorded on sowthistle, cheeseweed, common groundsel or beavertail cactus. Over all sites the mean number of adults recorded was largest on cotton wood (Table 1). In Redlands, more adults were found on hibiscus, oleander, Valencia orange, photinia, euonymus, ligustrum, cottonwood and cherry than in Piru or Pauma (results not shown). In Piru and in Redlands, more adults were recorded on cotton wood than on any other host plant species (t=59.75, *P*<0.00001 and t=72.05, *P*<0.00001 respectively). In Pauma, most adults were recorded on cotton wood, but these did not differ significantly from sycamore and grape (t=63.61, *P*<0.00001). Because of unequal variances Kruskal Wallis was used for these analyses (results not shown).

The data on the immature GWSS were collected as small, medium and large GWSS nymphs. For the purpose of these preliminary analyses the stages were added to present one number per host plant per observation at each location. The number of GWSS nymphs at the different locations changed through the season. From April though June, significantly fewer nymphs were recorded in Redlands when compared to Pauma and Piru in the same period (unequal variance: Kruskal Wallis: t=10.04, *P*=0.0066) (Fig. 1c). From Late July through October, significantly fewer nymphs were recorded in Piru, when compared to Redlands and Pauma in the same period (unequal variance: Kruskal Wallis: t=7.78, *P*=0.0204) (Fig. 1b). No nymphs were recorded on common groundsel. Over all sites the mean number of nymphs recorded was largest on cottonwood, followed by significantly lower numbers on grape, crape myrtle, and Valencia orange (Table 1). No differences were found when comparing numbers of nymphs per host plant species between the locations (results not shown). In Piru, most nymphs were recorded on cottonwood, followed by grape and citrus (t=70.3, *P*<0.00001). In Redlands, most nymphs were also recorded from cottonwood, followed by grape and crape myrtle (t=72.49, *P*<0.00001). In Pauma Valley, most nymphs were found on cottonwood and grape, followed by crape myrtle and Valencia orange (t=68.92, *P*<0.00001). Because of unequal variances Kruskal Wallis was used for these analyses (results not shown).

The recorded numbers of generalist predators present per location include lady beetles, spiders and lacewings. Less frequently praying mantis, assassin bugs, robber flies, scorpion flies and syrphid flies were recorded. The numbers of foraging parasitoids (*Gonatocerus* sp) were also recorded per plant. These data have not yet been analyzed. On June 30, July 1-2, August 10-12, September 28-30 xylem fluids samples were taken from all host plants except oleander, amaranthus, ivy, sowthistle, common groundsel, cheeseweed, lambsquarter, honeysuckle, primrose and beavertail. These species were omitted because experience has shown that they do not comply with the technique used for xylem extraction, rendering the sampling impossible (Brodbeck, personal communication). With the use of a nitrogen gas pressure chamber, 150-600µl was collected per plant and frozen for storage. The xylem samples await analyses on their chemical composition in Florida. The GWSS fecundity and feeding rate on a selection of the host plants listed in table 1 is being studied in University of Florida, NFREC-Quincy.

CONCLUSIONS

The data thus far indicates that the most eggs, nymphs and adults are not necessarily recorded on the same plant species as has been reported before (Brodbeck et al. 1999). In this study the only host plant used frequently in all life stages is cotton wood. On grape and crape myrtle nymphs and eggs are frequently recorded, while photinia, cherry and sycamore frequently

hosted egg masses but not the other life stages. The suitability of the host plants for these GWSS life stages may be linked to the chemical composition of the xylem fluids (Andersen et al. 1989, 1992, Brodbeck et al. 1990, 1993, 1995, 1996, 1999), data for which will be provided by the xylem analyses. Sowthistle, common groundsel, lambsquarter, cheese weed, primrose and beavertail were not hosting large GWSS numbers, if any, and may be discarded or replaced for next season.

This season, the location seems to influence the size of GWSS egg masses (larger egg masses in the south), survival (lower in the south) and parasitism (lower in the south). The underlying factors may be related to temperature and humidity which have been recorded but have not been correlated to the findings yet. The major difference between the coastal and inland locations at similar latitude is the number of second generation adults, and all life stages from the second generation are responsible for most of the location differences. Aside from the egg masses, there are no obvious differences in the other life stages recorded in the coastal and southern location.

Further conclusions cannot be drawn without the data that is still being taken in the fecundity and feeding studies and the chemical xylem composition of the host plants. For full understanding of the climatic influences behind these observations, multiple year data are needed and need to be analyzed for temporal and spatial differences, for which two additional years of funding will hopefully be forthcoming from the CDFA.

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FUNDING AGENCIES

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EXPLORATION FOR FACULTATIVE ENDOSYMBIONTS OF SHARPSHOOTERS

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ABSTRACT
Glassy-winged sharpshooters (GWSS) were collected in California and several states in the southeastern United States in 2002 and 2003 to search for pathogenic or beneficial endosymbiotic bacteria of these insects. Various tissues were examined for the presence of bacteria by PCR: hemolymph, eggs, and bacteriomes. A subset of hemolymph and egg samples were cloned and sequenced based on unique digest patterns of their extracted 16s rDNA, or analyzed by restriction digest patterns of sample compared to known bacterial DNA. Most cloned sequences were identified as *Baumannia* (one of the primary symbionts of GWSS), and *Wolbachia* (a common secondary symbiont in a majority of insect taxa investigated). In addition, we isolated bacteria that were most closely related (by 16S rDNA sequence) to the following genera: *Acinetobacter, Stenotrophomonas, Pseudomonas, Burkholderia.* All are common bacteria that are found in soil, water, or plant surfaces, and also in insect guts or surfaces.

INTRODUCTION
We have surveyed populations of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, for bacterial symbionts that might be exploited to manipulate the biology of this insect vector of *Xylella fastidiosa (Xf)* (Purcell and Feil 2001). Pathogens or other microbial associates of GWSS have not been employed to date as biological control agents or contributors to the control of these pests largely because none are known, although some efforts to discover viruses of GWSS have been made. Although endosymbiotic bacterial associates of leafhoppers are little-understood and unexploited to date, their potential importance is well worth exploring. The first step has been to look for and identify any naturally occurring bacteria in GWSS populations from a wide geographical range.

Of particular interest to us in this study were bacterial associates that are facultative (also referred to as "secondary"), i.e., that occur in some individuals or populations but are not required by their hosts; and that could be introduced into, or augmented in pest populations. We use the term symbiont here in the biological sense of "living together" and do not imply mutual benefit (Douglas 1994). Facultative bacterial associates have been described in a variety of homopterans including leafhoppers (Swezy and Severin 1930, Schwemmler 1974, McCoy et al. 1978, Purcell et al. 1986). The only leafhopper facultative symbiont studied in some depth is BEV, a bacterium that occurs in *Euscelidius variegatus* in France, but apparently not in California (Purcell et al. 1986). Uninfected females of *E. variegatus* inoculated with cultures of BEV transmitted the bacteria transovarially ("vertically") to their offspring, with resulting deleterious effects (Purcell et al. 1986, Purcell and Suslow 1987). This bacterium could also be transmitted horizontally between leafhoppers feeding on the same plant; hence it could persist in the population in spite of its negative fitness effects.

It is clear from our studies of facultative bacteria in aphids (Chen et al. 2000, Montllor et al. 2002) as well as from the study of BEV, that endosymbiotic associations are complex and have critically important effects, both positive and negative, on the physiology, population biology and vector potential of their hosts. Some of the most extensive studies on the effects of facultative symbionts on insect hosts involve *Wolbachia*, a transovarially transmitted bacterium that occurs in 20-76% of investigated insect species (Weeks et al. 2002) with a range of interesting effects (e.g.,Werren 1994, Stouthammer et al.1999). *Wolbachia* has recently been described from GWSS (Moran et al. 2003), though its effects remain unknown. Although *Wolbachia* has "helped raise the awareness of the potential contribution of endosymbionts...it is important not to discard other alternatives" (Weeks et al. 2002). Our approach was to investigate whether other alternatives existed for GWSS.

OBJECTIVES

1. Survey glassy-winged sharpshooter and other sharpshooters in California and the southeastern United States for facultative bacterial endosymbionts and determine by DNA sequencing the identity of any bacteria discovered.
2. Depending on type of microorganism and relative frequency in surveyed insects, select candidate symbionts to determine biological effects on GWSS.

RESULTS

We collected GWSS from various locations in California and in Louisiana and Florida in spring and summer 2002. In June 2003 we collected GWSS from Louisiana, Mississippi, Alabama and Florida. Four other species of sharpshooter were also collected in California in summer 2002 and fall 2003. Some field collected GWSS from selected locations were brought back to the lab and caged together for one to several weeks in order to facilitate exchange of any potentially horizontally transmitted facultative symbionts. In several cases, long-term lab colonies were established from field populations, and could be repeatedly sampled. Laboratory-reared GWSS were also obtained from the California Department of Food and Agriculture rearing facility in Bakersfield, California on several occasions in 2003.

DNA from three types of tissue from sharpshooters collected in 2002 and 2003 were extracted: hemolymph, eggs, and bacteriocytes. Over 400 extractions have been made and analyzed for bacterial DNA. Hemolymph is known to contain bacterial endosymbionts in aphids (e.g., Chen et al. 1996) and leafhoppers (e.g., Purcell et al. 1986) and is a logical place to sample. Approximately 2- 4 uL of hemolymph was removed by puncturing the abdomen with a glass needle, and was then added to 20 uL phosphate buffered saline (PBS) and stored frozen until analysis. After extraction, we amplified the DNA of the 16S ribosomal DNA with "universal" bacterial primers, digested any bacterial DNA with restriction enzymes, and looked for different patterns that might indicate the presence of more than one type of bacteria. A subset of bacterial 16S rDNA was cloned in *E.coli*, reanalyzed with restriction enzymes (e.g., Table 1), and sequenced if deemed appropriate. This procedure was also applied to eggs (dissected from gravid females or removed from leaves after being laid) in which we expected to find any vertically transmitted endosymbionts, such as the primary symbionts, *Baumannia,* but perhaps other symbionts as well.

Forty-five percent (126/281) of hemolymph samples from all localities tested positive for bacterial 16S rDNA by PCR. Twenty-six individuals of another four species of sharpshooters from California were also tested for bacteria in hemolymph, of which five (19%) were positive by PCR. We have not yet analyzed these further. DNA from a total of 25 GWSS tissue samples from 17 individuals was chosen for cloning, and 19 produced multiple transformed *E. coli* colonies with bacterial 16S rDNA inserts. DNA from 45 of these colonies was chosen for sequencing, and others were identified by restriction digest analysis. The most common sequence was identical to that of *Baumannia*, a bacteriome-associated symbiont of the GWSS (Moran et al. 2003) (Table 1). Like other bacteriome inhabitants, *Baumannia* is presumably transovarially transmitted from mother to offspring via hemolymph (Buchner 1965). *Wolbachia*, a commonly found facultative symbiont of many insects, including GWSS (Moran et al. 2003), was also cloned or commonly found facultative symbiont of many insects, including GWSS (Moran et al. 2003), was also cloned or otherwise identified from hemolymph and eggs of California, Florida and Louisiana GWSS. In addition, we surveyed extracted DNA that was positive for 16S rDNA for *Wolbachia* by PCR. *Wolbachia* has been described from GWSS (Moran et al. 2003), but its prevalence and the existence of strain differences has not been documented. We found *Wolbachia* in 10% (8/84) of hemolymph samples and 59% (19/32) of egg samples. These figures are probably conservative, and indicate that *Wolbachia* is a very common bacterium associated with GWSS. *Baumannia* was amplified from 67% (60/89) of hemolymph samples by PCR.

Table 1. Cloned bacterial DNA from GWSS tissue samples. Bau=Baumannia; Wol=Wolbachia, Aci=Acinetobacter; Pseu=Pseudomonas; Burk=Burkholderia.

Collection location (sample / no. clones sequenced or digested)	GWSS tissue	16s rDNA identity of inserts (by sequencing or restriction digest analysis)
Bakersfield	Hemolymph Eggs	Bau, Wol, Aci Wol, un-id
CDFA	Hemolymph Eggs	Bau, Wol, un-id
Louisiana State Univ	Hemolymph Eggs	Bau, Sten, Pseu Bau
Crestview FL	Hemolymph Eggs	Bau, Wol, Aci, Pseu Bau, Wol
Pearl River LA	Hemolymph Eggs	Bau, Wol, un-id Bau, Wol, Burk

Although *Baumannia* and *Wolbachia* were the most common bacteria found, a few other 16S rDNA of bacteria not previously described from GWSS were also cloned from GWSS samples (Table 1). Some samples are still being analyzed to determine the identity ("un-id" in Table 1) or close relationship of the bacteria represented. Among those isolated were bacteria with identity similar to *Acinetobacter*, *Stenotrophomonas*, *Pseudomonas* and *Burkholderia.* All are aerobic γ-Proteobacteria, and not uncommon as environmental contaminants and nosocomial pathogens (e.g., Towner et al. 1991, Ribbeck et al. 2003). However, *Acinetobacter* and *Stenotrophomonas* have also been isolated from ticks and fleas (Murrell et al. 2003); and *Stenotrophomonas*, among other bacteria, was isolated from the guts of ants, where it was presumed to provide nutrients and to be passed to offspring (Jaffe et al. 2001). *Stenotrophomonas* was also described as an endosymbiont of a fly (Otitidae), which did not develop properly without its complement of bacteria (Wozniak and Hinz 1995). *Burkholderia*, a pseudomonad, was isolated from termite guts (Wertz et al. 2003), and was able to colonize a variety of aquatic invertebrates both externally and internally (McEwen et al. 2001).

We did not detect any bacteria in PBS buffer alone. Bacteria were detected in 4 of 12 buffer samples that were pipetted onto the outside surfaces of 12 different insects. We were only able to clone one of these DNA samples because subsequent PCRs of the other three were negative for 16S DNA. The cloned sample contained 16S DNA similar to that of *Pseudomonas*, *Acinetobacter*, and *Methylobacterium*. It is not yet possible, therefore, to determine whether *Acinetobacter* and *Pseudomonas* cloned from hemolymph samples came from the insect surface, the hemolymph, or both.

CONCLUSIONS

A wide-ranging search for secondary symbionts of the GWSS did not identify good candidates for studies on biological effects on this insect. Some bacteria we identified were possibly from insect external surfaces. The prevalence of a *Wolbachia* species, and the well-known importance of *Wolbachia* to other insect hosts make it the best candidate to pursue in further studies.

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EFFECTS OF SUBLETHAL DOSES OF IMIDACLOPRID ON VECTOR TRANSMISSION OF *XYLELLA FASTIDIOSA*

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ABSTRACT
A computer-monitored flight mill was developed to study the effects on insect flight of sub-lethal dosages of soil-applied imidacloprid (Admire 2F, 21.4% AI) to glassy-winged sharpshooters (GWSS) in laboratory cages. Adult sharpshooters were glued to a 10 cm radius plastic arm that rotated on a pivot. The rotations per minute were recorded and tabulated by computer. The range of distances flown on flight mills by adult GWSS not exposed to insecticide treatment (negative controls) ranged from 8 m to 6,843 meters and averaged 3,853 m for males and 2,537 m for females. Over 90% of males and females flew at least 60 m ("fliers") during the 6-12 hour flight trials. More than 9 % of total distances flown by individual fliers occurred within 4 hours. Imidacloprid at sub-lethal dosages (9% mortality in 24 hours vs. 3% of untreated controls) that inhibit feeding did not reduce flight performance significantly, but dosages that killed 33% of the GWSS in 24 hours reduced flight in the surviving insects. Insects that had fed on insecticide-treated plants for 24 hours flew much less (fewer fliers), yet among those that did fly, the differences were not statistically significant. At 3.2 mg imidacloprid in 500 g soil, on average, one-third were killed after 24 hours, and less than 50 % of the survivors flew. However, there were occasional "outliers" that could fly just as well as, or sometimes more than, the control insects. Whether these individuals were resistant to imidacloprid or survived and flew as a result of uneven uptake of the insecticide by different replicate plants was not clear. There were no significant differences in flight distances of GWSS exposed to a dose of 0.1 mg in 500g soil.

INTRODUCTION
The systemic insecticide imidacloprid (Admire 2F, Bayer Co., Kansas City, MO) has been used to control glassy-winged sharpshooter (*Homalodisca coagulata*, GWSS) in citrus and grapes, mainly as a killing agent (Bethke et al. 2001). The main effect of insecticides in reducing the spread of Pierce's disease is to decrease the numbers of insects entering and remaining in vineyards. But beyond the numbers of GWSS, disease spread also depends on the level of infectivity of GWSS with *Xylella fastidiosa*, vector transmission efficiency to grape, and movements of the vector from plant to plant (Purcell 1981). GWSS movements from vine to vine should be especially important if this is the main mode by which GWSS establishes new infections of grape, as circumstantial evidence suggests (Perring et al. 2001; Purcell and Saunders 2001). Sub-lethal (low lethality) dosages may persist in treated crops longer than highly lethal dosages, as plant growth dilutes insecticide concentrations and the insecticide deteriorates to less toxic or non-toxic forms. Identifying the effects of sub-lethal dosages on the behavior of a plant disease vector is especially important because non-lethal doses of insecticide may repel some insects and increase plant-to-plant movements, leading to increased disease spread by surviving vectors. Our previous studies suggested that imidacloprid does not repel the GWSS or promote their small scale plant-to-plant movement.

Our objectives were to establish the effects of sub-lethal dosages of imidacloprid on GWSS transmission efficiency and movement. As we previously reported (Purcell 2003), systemic imidacloprid (soil applications) in grape reduced GWSS transmission of *X. fastidiosa* to grape, but the effects might have been mostly due to insect mortality rather than by affecting GWSS feeding behavior in such as way as to reduce vector transmission. Dosages that did not kill more than 10% of GWSS significantly reduced feeding by GWSS, but imidacloprid did not repel GWSS or blue-green sharpshooters in lab trials in which a documented repellent, Surround, did repel sharpshooters from plants (Purcell 2003).

We tested various dosages of imidacloprid that caused reduced GWSS feeding to determine the effects of the insecticide exposure on the flight performance of GWSS on flight mills. Computer-monitored flight mills have been used to study flight performance in other leafhoppers (Gorder 1990; Taylor et al. 1992), and we adopted a previously described flight mill design (Gorder 1990; Schumacher et al. 1997) to assess the flight performance of GWSS with or without exposure to imidacloprid treatments of grape. Flight mill performance usually requires about 30% of the power required for free flight (Riley et al. 1997), so flight mills underestimate free flight distances.

OBJECTIVES
1. Understand basic performance characteristics of GWSS flight.
2. Determine the effect of various doses of imidacloprid on the flight performance of GWSS in the context of Pierce's disease epidemiology.

RESULTS

Objective 1. Understand the Basic Characteristics of GWSS's Flight.

Flight mills were constructed as outlined by Schumacher et al. (1997), with slight modification. The rotating flight mill arm was a 20cm plastic drinking straw rotating on a jewel bearing fitted with a steal shaft. Custom computer software counted the number of revolutions in successive 60-second intervals and generated data on flight distance, duration, and velocity. For each trial, 3 replications of 4 to 10 GWSSs per cage were allowed to feed on grape for 24 hours. The prothorax of each insect was glued to a standard insect pin using water-soluble Styrofoam glue, and the insect pin (with the insect attached) was then inserted into the arm of the mill. Flight trials lasted for 12 hours, later reduced to 4 hours, during the day. GWSS were classified as "fliers" if they flew a total distance of 100 rotations (63 m) and "non-fliers" if they failed to complete 100 rotations. Table 1 summarizes the flight mill performance of GWSS from untreated plants. Males consistently flew longer and more frequently than females (Figure 1), so data for males only (Table 2) were summarized for comparisons of GWSS from treated and untreated grape. Figure 2 illustrates a typical flight profile for GWSS males from untreated (Figure 2A) and high dosage plants (Figure 2B).

Objective 2. Examine the Effect of Various Doses of Imidacloprid on the Flight Performance of GWSS in the Context of Pierce's Disease Epidemiology

To quantify the effects of sub-lethal dosages of Admire on GWSS flight performance, we measured the flight performance of insects exposed to both treated and untreated grape vines. Imidacloprid treatments were dilutions of a standard 3.2 mg in 500 g of soil. Dilutions used were 1/4, 1/8, 1/16, 1/32 of the standard dose; controls were untreated vines. The plants were allowed one week for pesticide uptake before caging the insects on them for 24 hours and then monitoring their flight mill performance. The 1/32nd dilution caused 9% mortality over a 24-hour period, compared to controls (3%) and did not significantly reduce total distance flown. A higher dose (1/4 of standard) did kill significantly more GWSS (33%) within 24 hours and reduced the numbers of surviving insects classified as "fliers", but some individual GWSS from the 1/4th dosage plants flew as well as those from untreated plants (Table 2). This may have been because of physiological variation among individuals or the amount of imidacloprid taken up by plants on which the insects had fed. We collected and froze xylem saps to compare imidacloprid concentrations from each plant to the flight performance of the GWSS that fed on them before flight mill assays but have not yet analyzed these samples for imidacloprid content.

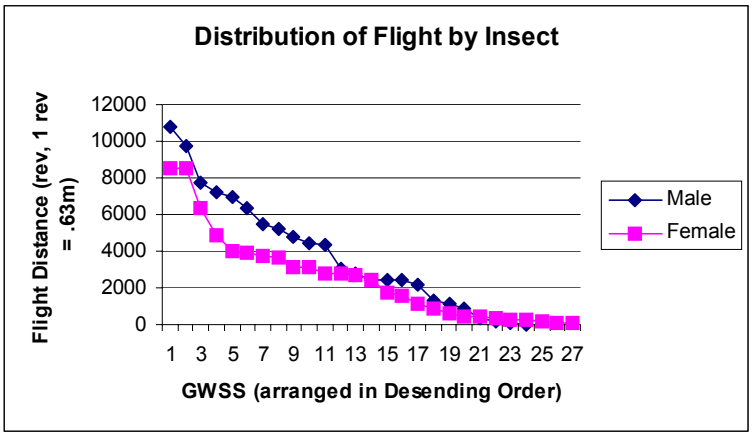

Figure 1. The flight distances of GWSS male (diamonds) and female (squares) from untreated plants.

Table 1. Flight mill performance of GWSS from untreated grape (control).

Performance characteristics		Range	Average	Stand. dev.
Total Revolutions	Males	12-10,826	3,853	3,085
	Females	72 - 8,557	2,537	2,410
Total flight events	Males	17-200	75	42
	Females	13-207	79	57
Distance of longest flight event				
	Males	6-1258 meters	358 m	359
	Females	6-495 m	149 m	140
Average distance per flight event				
	Males	6-178 m	70 m	46.9
	Females	6-151	37	40.8

Table 2. Mortality and flight performance of GWSS males after a 24-hour exposure to untreated grape or grape with imidacloprid applied at 1/4th or 1/32nd of a standard dose (3.2 mg/500 g soil) 10 days previously.

Performance characteristic (Males Only)		Sample size	Range	Average *	Stand. dev.
Mortality	1/4th dose	57	0- 100%	33% a	0.34
	1/32nd dose	48	0 - 25%	9% b	0.09
	untreated	48	0 - 20%	3% b	0.08
Percentage of surviving non-fliers					
	1/4th dose	38	0 - 100%	59% a	0.38
	1/32nd dose	44	0 - 22%	7% b	0.09
	untreated	46	0 - 20%	3% b	0.08

*Numbers in a column followed by the same letter were not significantly different using chi-squared with Yates' correction and ANOVA.

The flight performance assays of GWSS exposed to 1/8th and 1/16th dilutions of the standard dosage of imidacloprid are still in progress. Preliminary indications are that the 1/8th dilution may reduce average flight activity but with some individuals flying as far as fliers from untreated plants.

Unreported Results that were Pending Last Year.

The effects of the insect-repellent kaolin clay (Surround) and Admire applied to potted grapevines were assessed in cages for possible repellency effects to GWSS and BGSS (Purcell 2003). In general Surround was repellent, whereas Admire was not. The test plants used in these behavioral experiments were saved for diagnosis for PD, as all sharpshooters used in the experiments had been exposed to plants infected with *X. fastidiosa*. Unfortunately, transmission rates in all treatments (including untreated controls) were too low (3% per plant for GWSS, 9-21% for BGSS) to be of value in assessing the effects of Admire or Surround applications on the vector transmission of *X. fastidiosa* where the insects had a choice of treated vs. untreated plants. This lower than normal transmission rate was probably due to low populations of *X. fastidiosa* in the PD-grapes used for acquisition feeding.

CONCLUSIONS

GWSS flew on flight mills for up to 4. 2 miles (6.8 km), averaging over 1.5 miles in a 4 hr period. Soil-applied imidacloprid (Admire) dosages that caused 33% mortality during a 24-hr exposure to treated plants reduced average flight performance of surviving GWSS, but some of the insects that survived this exposure flew almost normally. Dosages that caused about 10% mortality and that have been shown to drastically reduce GWSS feeding did not significantly reduce flight on flight mills. Admire treatments probably reduce long distance movements of GWSS from treated crops having sap concentrations of imidacloprid that kill at least 30% of the GWSS within 24 hours.

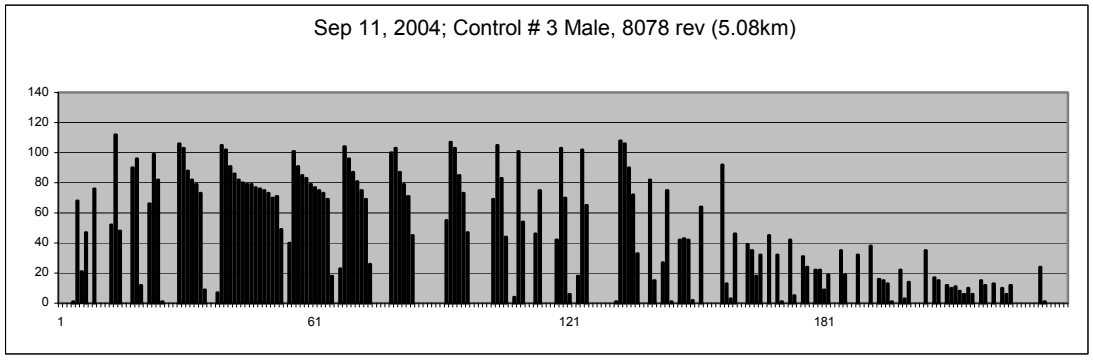

Figure 2A. Flight (flight mill rotations per minute) of a control GWSS (no insecticide); horizontal axis = minutes.

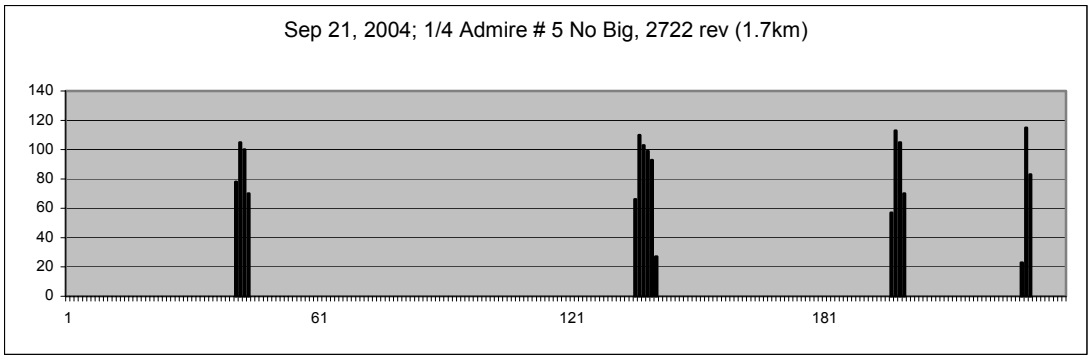

Figure 2B. Flight of a surviving GWSS fed on grape treated with 1/4 of standard dose. Note flights are fewer and shorter than untreated insects.

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A NOVEL METHOD TO INDUCE OVIPOSITION IN THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT
Gravid *Homalodisca coagulata* females were induced into ovipositing a significantly greater proportion of their eggs 24h after desiccation treatment with a directed flow of warm air (40°C, 5.0 meters per second for 15 m) compared to untreated females. Treated and untreated females oviposited 54.5% and 28.2% of their eggs, respectively, regardless of host plant.

INTRODUCTION
Accidental introductions of *H. coagulata* into regions of California have prompted researchers to begin a classical biological control program using egg parasitoids in the genus *Gonatocerus* (Jones 2001). Initiation and maintenance of large cultures of *H. coagulata* for egg production for culture of *Gonatocerus* parasitoids is difficult and time consuming because few host species adequately support all life stages of *H. coagulata* (Brodbeck et al. 2004). Currently, augmented releases of *Gonatocerus* parasites are an important component of long-term management of *H. coagulata* in California.

The phenomenon of death stress oviposition was first reported by DeCoursey and Webster (1952) who indicated that a variety of chemical agents, including pesticides, could produced various levels of stress to gravid female mosquitoes *Ochlerotatus sollicitans* (Walker) and gravid Angoumois grain moth, *Sitotroga cerealella* (Oliver). Individuals that were stressed deposited a greater amount of eggs than untreated controls.

One of the objectives of our research project is to determine the behavioral and physiological mechanisms associated with the overwintering of *Gonatocerus* eggs parasitoids, an important natural enemy of *H. coagulata*. Efficient acquisition of even-aged cohorts of *H. coagulata* eggs is crucial to this project. For nearly 20 years, our research group has been involved in the study of many life history characteristics of *H. coagulata*, including oviposition behavior.

OBJECTIVES
The main objective of this study was to determine and manipulate the environmental conditions conducive to inducing oviposition of gravid *H. coagulata* females.

RESULTS
Twenty gravid females were field-collected from crape myrtle, *Lagerstroemia indica* L. by sweep net. Ten females were placed immediately into a cage that was provisioned with either one three-week old cotton plant, (*Gossypium hirsutum* (L.) 'Deltapine 88'), or one glabrous soybean plant, (*Glycine max* (L.) 'D90-9216'). Ten females were stressed with a direct flow of warm air (40°C, 5.0 meters per second) for 15m (Fig 1). After airflow treatment, females were placed into a cage with a plant as described previously. Plants were examined for egg masses the next morning. Females were dissected and numbers of mature, chorionated oocytes in the lateral and median oviducts were counted. Tests with each host plant were replicated three times. Host plant effects on oviposition were analyzed by ANOVA (SAS 1990). We defined the experimental unit as total eggs per plant, as we could not accurately quantify eggs per female. Paired comparison t-tests were used to compare the differences between the total eggs, number of eggs oviposited, and of mature chorionated oocytes not oviposited between treated and control females.

Host plant had no effect on oviposition of stressed ($F = 0.84$; $df = 1, 4$ $P < 0.42$) or unstressed females ($F = 0.03$; $df = 1, 4$ $P < 0.88$). Data from the six replications were then combined for t-test analysis. Field-collected gravid *H. coagulata* oviposited a significantly higher proportion of their eggs following stress treatment compared to unstressed controls (Table 1.). Targeted dissections indicated that stressed females had fewer chorionated oocytes within reproductive structures than females that were not stressed.

Figure 1. Airflow apparatus used to induce desiccation stress in gravid female *H. coagulata*.

Table 1. Means (±SE) of number of eggs oviposited and or retained by stressed and unstressed gravid *H. coagulata*. Values across rows followed by different letters are significantly different; P<0.05.

	Stressed	Unstressed	Pr> |t|
Mean±SE[a]			
Proportion of eggs oviposited	54.4±4.4a	28.2±5.3b	0.002
Eggs oviposited per female	13.7±2.3a	5.9±1.2b	0.015
Total oviposited + mature oocytes	194±18.9a	187.2±17.1a	0.696

[a]*n*=six replications

CONCLUSIONS

A broad ovipostional host range may not necessarily be disadvantageous to the neonates of *H. coagulata*, as we have recently documented adaptations that allow the immature stages to efficiently relocate to suitable hosts (Tipping et al. 2004). Stress-induced oviposition thus appears consistent with both the reproductive physiology and the nutritional ecology of *H. coagulata* due to the inability of females to reabsorb oocytes and the high vagility of immatures.

The phenomenon of death stress oviposition, or induced oviposition, in *H. coagulata* can be a valuable tool for researchers who require large numbers of uniform aged eggs essential for nymphal development studies. Additionally, this technique can be useful for maintaining cultures of *Gonatocerus* parasitoids. Finally, collection of many egg masses in a short period of time may also be instrumental in the creation or augmentation of existing cultures of *H. coagulata*.

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OVERWINTERING BIOLOGY OF THE GLASSY-WINGED SHARPSHOOTER AND GONATOCERUS ASHMEADI

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Reporting period: The results reported here are from work conducted June 2003 to September 2004.

ABSTRACT
The Glassy-winged Sharpshooter, *Homalodisca coagulata* (Say), is found throughout southeastern US and regions of California. It has 2 distinct generations per season. The majority of adult females overwinter in a reproductive diapause. Targeted dissections of female *H. coagulata* reared at a photoperiod of 13:11 at 23-29^{o}C indicated all females were in reproductive diapause. Seventy-five percent of females reared at a photoperiod of 13.5:10.5 at 23-29^{o}C entered reproductive diapause, perhaps indicating that photoperiod can be modified by temperature as the trigger responsible for physiological changes associated with reproductive diapause. Diapause can be broken by placing females at a 11:13 photoperiod (15-17° C) for 21d followed by exposure to mid-summer environmental conditions. Additionally, parasitism of *H. coagulata* eggs by *Gonatocerus spp*. peaked sharply in early April 2004 and remained at 100% until the last week of September 2004. Finally, short-day photoperiod did not effect development or host seeking behavior of *G. ashmeadi*.

INTRODUCTION
The overwintering biology of the Glassy-winged Sharpshooter, *Homalodisca coagulata* (Say) is an important component of seasonal population dynamics. In the southeastern US, host plant preferences of adult *H. coagulata* during spring, summer, and fall months are predictable and intimately associated with nutrition (Mizell and French 1987, Brodbeck et al. 1995). Mixed hardwoods and citrus are the preferred overwintering hosts for *H. coagulata* in its endemic and parts of its introduced range in California, respectively (Pollard and Kaloostian 1961, Blua and Morgan 2003). In most years, females break diapause during early to mid-March and begin to oviposit on a variety of plants (Turner and Pollard 1959). Presently, the physiology associated with the overwintering biology of *H. coagulata* is poorly understood.

Gonatocerus ashmeadi Girault is one of several egg parasitoids that are key natural enemies of *H. coagulata*. Little is known about their overwintering biology. Lopez et al. (2004) report *G. ashmeadi* could potentially overwinter in the eggs of their host. A greater understanding of the life history of *G. ashmeadi* is essential to maximizing their utility as classical biocontrol agents.

Diapause is loosely defined as a temporary inactivation or reduction of one or several physiological processes triggered by an environmental cue (Lees 1966). Arthropods enter diapause to survive adverse environmental conditions (Masaki 1980). Photoperiod is often the primary cue that triggers physiological changes associated with diapause, however, other environmental factors including temperature and nutrition can have a modifying effect. In the southeast US, *H. coagulata* overwinters primarily in the adult stage (Turner and Pollard 1959). However, 5th instar nymphs and viable eggs can occasionally be found in north Florida during the winter months.

OBJECTIVES
The environmental conditions that are responsible for initiation and cessation of reproductive diapause in *H. coagulata* are a major focus of this research project. Additionally, the effects of photoperiod and temperature on the development and behavior of *G. ashmeadi* were also investigated.

RESULTS
Because diapausing individuals are unidentifiable from non-diapausing individuals, we have developed and refined a protocol for targeted dissections to accurately determine the reproductive status of female *H. coagulata*. Leafhoppers were immobilized with gentle pinch to the head, placed in a paraffin filled dissecting dish and viewed under a stereoscope. The wings and telson were carefully removed with fine jewelers forceps followed by small incisions along the pleural membrane of the abdomen. The abdominal terga were then removed to facilitate examination of four Malpighian tubules, which lie dorsally in loops above the mid and hindgut. Fat body was generally concentrated in the first through fourth abdominal segments. Ovarioles were examined after portions of the gut tract were teased out of the body cavity. Ovarioles, ova, fat body, and Malpighian tubules were rated on the scale described in Table 1.

Cohorts *H. coagulata* neonates were reared to adult on lemon basil, *Ocimum basilicum* L. 'Lemon', glabrous soy, *Glycine max* (L.) 'D90-9216', and cotton, *Gossypium hirsutum* L. 'Deltapine 88' in environmental chambers programmed with photoperiods of 13.5:10.5, or 13:11 at 23-29^{o}C. Females were dissected and rated as described previously, 15-28d post eclosion. Additionally, cohorts of *H. coagulata* were reared under ambient lighting in a greenhouse during summer and winter months and dissected. Targeted dissections revealed that all female *H. coagulata* reared under the 13:11 photoperiod were in reproductive diapause when compared to individuals reared in winter conditions (Table 1). Dissections of females reared under the 13.5:10.5 photoperiod indicated that 25% (5 of 20) were reproductively active when compared with cohorts reared under early summer conditions (Table 1).

Female *H. coagulata* in reproductive diapause can be manipulated into becoming reproductively active. Cultures of overwintering *H. coagulata* were maintained in screen cages in a greenhouse at ambient light and temperatures. On January 20, 2004, cohorts of leafhoppers were placed into an environmental chamber with a programmed photoperiod of 11:13 (15-17°C) for 21d. They were then moved to a greenhouse set for summer conditions (14:10, 32°C). After 12-14d, brochosomes were observed on the forewings of many of the females. Egg masses were usually present two days later. Five cohorts of leafhoppers were treated as described previously with the same results.

A glabrous soy plant with approximately 20 *H. coagulata* egg masses was exposed to a culture cage of *G. ashmeadi* for 24h. The plant was then placed into an environmental chamber programmed with an 11:14 photoperiod (26°C). Parasites were observed emerging from parasitized egg masses after 14d. The plant was removed and egg masses evaluated for parasitism. All eggs were parasitized and all adult *G. ashmeadi* had successfully eclosed. Two additional plants with egg masses were treated as described previously with similar results. Additionally, adult *G. ashmeadi* that eclosed in the chamber were provided with a new soy plant with approximately 15 *H. coagulata* egg masses. After 14d, adults were observed emerging from the egg masses indicating short-day photoperiod had no effect on their life history.

Single potted cotton or glabrous soy plants with *H. coagulata* egg masses were placed in the field on a weekly schedule beginning the first week of March 2004. After 15d all egg masses were checked for signs of *Gonatocerus* parasitoids. Seasonal parasitism peaked sharply in early April and fell sharply in late September 2004 (Table 1).

CONCLUSIONS

Examination of the ovarioles, ova, fat body, and Malpighian tubules can provide an accurate indication of the reproductive status of female *H. coagulata*. We conclude there is a critical photoperiod important for the initiation of reproductive diapause in *H. coagulata*. However, we have not determined the sensitive life stage to these diapausing inducing cues. We have also determined the environmental conditions important for the termination of reproductive diapause. Additionally, *G. ashmeadi* does not appear to modify its life history when reared under short-day photoperiods in an environmental chamber.

Since populations of *H. coagulata* are reproductively active in north Florida for a relatively short period of four months, overwintering and diapause play a critical role in population dynamics of these insects. Understanding environmental cues critical to reproductive diapause initiation and termination are also essential for researchers attempting to rear these insects throughout the year.

The photoperiod responsible for reproductive diapause of all female *H. coagulata* corresponds to August 24 in north Florida. During this time of year and several weeks later, temperature, rainfall, and host plant availability remain adequate for an additional generation of *H. coagulata*. We propose that this early seasonal reproductive diapause of *H. coagulata* is a life-history response to predation pressure by *Gonatocerus spp*. egg parasitoids.

Table 1. Results of targeted dissections of internal reproductive morphology of *H. coagulata* reared under several photoperiod and temperature regimes.

Photoperiod and Temperature[a]	*n*	Ovarioles	Ova	Fat body	Brochosomes
13.5:10.5 (Aug 5)	15	2	0	2.5-3	1
23-29°C	5	3	2	2.5-3	3
13:11 (Aug 24)	18	2	0	3	1
23-29°C	6	2	0	2.5	1
Greenhouse (May19-Jun29)	15	3	2	2.5-3	3
13h 13m – 14h 5m (photophase)					
31-37°C					
Greenhouse (Jan6-Feb26)	15	2	0	3	1
10h 16m – 11h 16m (photophase)					
16.7-27.2°C					

[a]Photoperiod and date for latitude of Tallahassee, FL.

Key:

Ovarioles
1=not developed
2=fully developed; no ova
3=fully developed with ova

Ova
0=none
1=single ova per ovariole
2=two ova per ovariole

Fat body
1=minimal
2=medium
3=heavy

Brochosomes (within Malpighian tubules)
1=small; tubule translucent
2=medium; tubule filled opaque white
3=large; tubule swollen opaque white

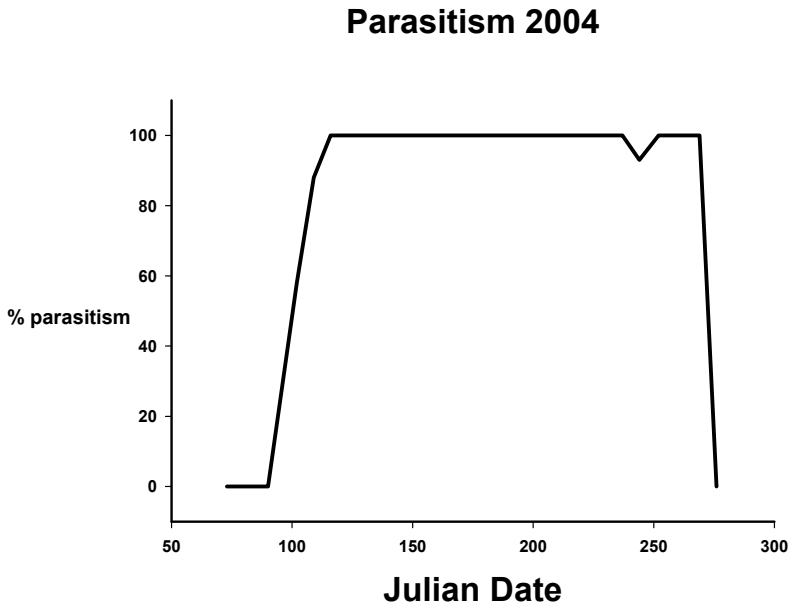

Figure 1. Seasonal parasitism of *H. coagulata* eggs by *Gonatocerus spp*. in north Florida.

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FUNDING AGENCIES

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EVALUATION OF BLUE-GREEN SHARPSHOOTER FLIGHT HEIGHT

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Reporting period: The results reported here are from work conducted from February 2004 to September 2004.

ABSTRACT
Flight heights of blue-green sharpshooters between vineyards and riparian zones were monitored at eleven sites in Napa Valley in 2004 using pole towers to position yellow sticky cards up to 24 feet. At 10 of the towers, nearly 90% of catches from March-September were made at 15 feet or lower. At one tower, however, a large number of BGSS were caught in the upper traps in early March. This tower's proximity to a Coast Live Oak (*Quercus agrifolia*) tree suggests that BGSS may reside at higher elevations in trees at some times of year.

INTRODUCTION
Where the blue-green sharpshooter (BGSS), *Graphocephala atropunctata,* is the primary vector of Pierce's disease (PD), control measures should be aimed at reducing the number of BGSS entering vineyards (4), especially early in the growing season. Early-season infections (March-May) are responsible for most chronic cases of PD (6, 9). Those infections resulting from BGSS feeding later in the growing season are not likely to result in PD, because most will be eliminated with normal pruning. This is unlike the situation with PD caused by glassy-winged sharpshooter (GWSS) feeding, where chronic infections may occur nearly year-round (1).

Vector control measures in the North Coast include the use of insecticides (4) as well as management of riparian plant communities to reduce the number of favorable BGSS breeding host plants (5).

Another method of reducing vector numbers is to block their flight into vineyards through the use of physical barriers. This could include the use of tall fences made with insect screening materials, as well as natural barriers created by planting dense stands of conifers or other non-host tree species. Both of these approaches are already being employed in a few vineyards in the North Coast, although there are currently no data to show their impacts. The use of barriers has also been suggested as a management tactic to keep GWSS out of vineyards (2).

For barriers to be effective, they would need to block the majority of BGSS from entering vineyards, since small numbers of insects can still lead to significant disease development (8). Unfortunately, little is known about the overwintering behavior of BGSS and its preferred winter plant hosts (7). Therefore, it is not clear how tall a barrier would need to be in order to be effective. Most trapping by both researchers and growers has been done from the ground at the 5-6 foot level. Monitoring of BGSS flight activity at higher elevations has not been reported.

This project addresses the question of BGSS flight height by installing and monitoring pole towers that can accommodate yellow sticky card trapping up to a height of approximately 24 feet.

OBJECTIVE
1. Evaluate the predominant flight height of blue-green sharpshooters entering vineyards from adjacent riparian habitats through the use of yellow sticky cards positioned at heights from 5 to 24 feet.

RESULTS
Eleven pole towers were installed and monitored in the Napa Valley in 2004. Towers were positioned along riparian zones adjacent to vineyards with a history of Pierce's disease. A diagram of a pole tower is shown in Figure 1. Towers were 25 feet in height, constructed from Schedule 40 PVC pipe. Yellow sticky cards were attached to clips on rope at the following heights: 24 feet, 20 feet, 15 feet and 10 feet. An additional trap at 5 feet was mounted on a stake.

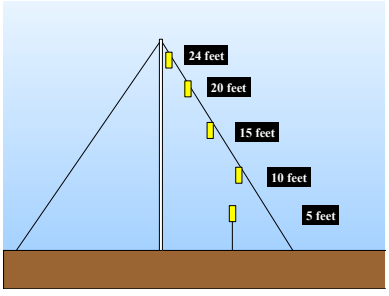

Figure 1: Pole tower diagram.

Eight towers were installed in February 2004; the remaining three were installed prior to March 9. Traps were monitored on a weekly basis through September and numbers of BGSS were recorded. Traps were replaced every two weeks or as needed.

Figure 2 shows the average numbers of BGSS trapped at various heights during the early season period of March-May. Figure 3 shows the average numbers of BGSS trapped at various heights during the entire trapping period of March-September. Figures 2 and 3 include results for all towers except #10, which will be discussed separately.

From March-May, each tower averaged 16.4 BGSS. Of these, 88.3% were caught at 15 feet or lower. For the entire season, each tower averaged 23.5 BGSS. Of these, 89.7% were caught at 15 feet or lower. The patterns of trap catches for the early part of the season and the full season were nearly identical.

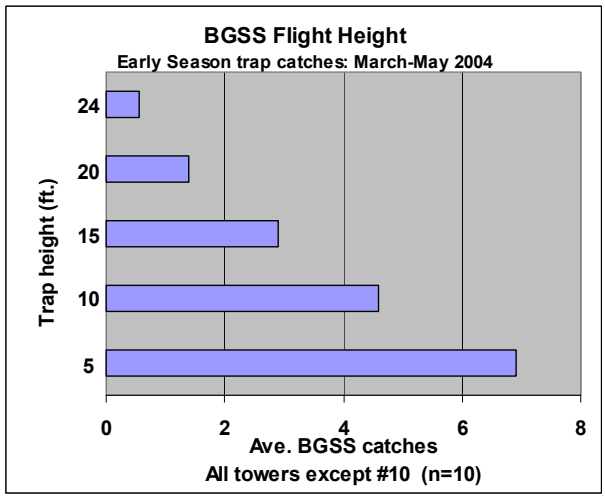

Figure 2.

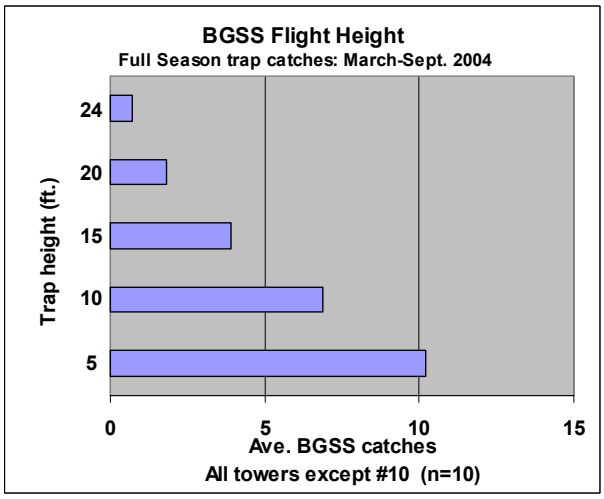

Figure 3.

These data suggest that a 15-20 foot high barrier could be effective at greatly reducing the number of BGSS entering vineyards. However, previous work with insecticides showed that even with 70-90% reductions in BGSS trap counts, the incidence of PD was not significantly reduced in vineyards planted with highly sensitive varieties (8). With a 10-15 foot screen barrier, the number of BGSS flying over the top could still result in significant amounts of PD in an adjacent vineyard.

Tower 10 had early season results very different than the others and is therefore considered separately. Figure 4 shows trap catches at Tower 10 during early March. Unlike the other towers, most BGSS were caught on the upper traps. However, for the rest of the season, the pattern of trap catches mirrored that of the other towers, albeit with greater numbers of BGSS (Figure 5).

Tower 10 was installed adjacent to a Coast Live Oak (*Quercus agrifolia*) tree, an evergreen species. Most of the other trees and shrubs in the vicinity of Tower 10 were deciduous species. In early March, these plants were still dormant or just beginning to bud out. A record heat wave in early March led to daily high temperatures of 70-85°F for nearly 2 weeks. The estimated flight threshold temperature for BGSS is 58°F (2). This unseasonable heat wave led to significant BGSS flight activity in early March as evidenced by elevated trap numbers at Tower 10 and others (data not shown).

The Coast Live oak tree adjacent to Tower 10 was apparently a preferred host plant at this time. If BGSS commonly reside in tall trees during the spring, then the effectiveness of barriers will likely be reduced. Additional studies are needed to better elucidate the early spring host preferences of BGSS in riparian zones, especially at higher elevations in the riparian canopy.

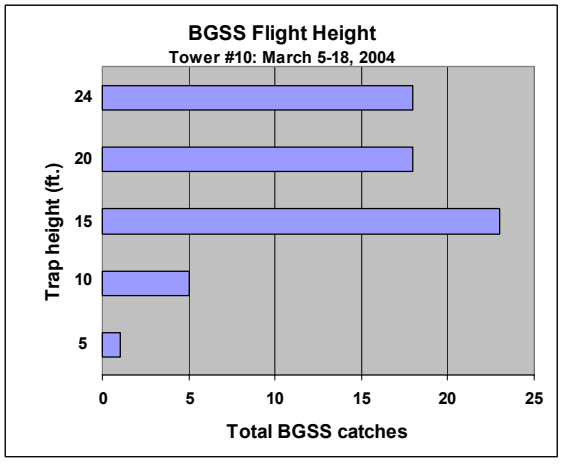

Figure 4.

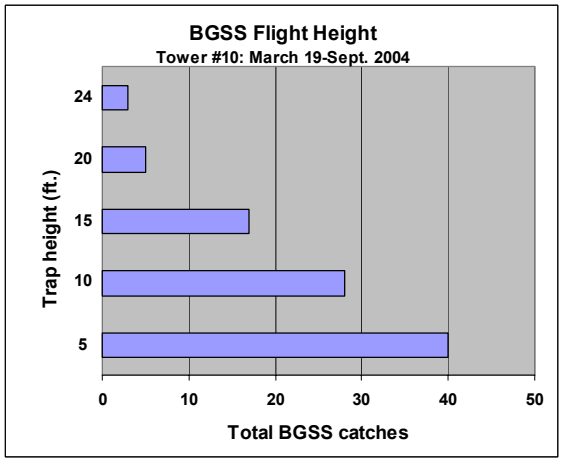

Figure 5.

CONCLUSIONS

Nearly 90% of the BGSS trapped in this study were caught on traps at 15 feet or lower. This suggests that barriers could have a significant impact on reducing the numbers of BGSS entering vineyards. However, this may not be enough to have a major impact on reducing the incidence of PD. In addition, results from one tower indicated that BGSS may reside in some trees early in the season. This could allow for higher than normal flight activity, allowing more BGSS to enter vineyards by flying over a barrier. The effectiveness of barriers at reducing the incidence of PD will likely depend upon the nature of the adjacent riparian plant community, its mix of host plant species and the number of tall host trees.

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REPRODUCTIVE BIOLOGY AND PHYSIOLOGY OF FEMALE GLASSY-WINGED SHARPSHOOTERS

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ABSTRACT
Female and male GWSS have been collected from July 2001 to September 2004 at monthly or bimonthly intervals from citrus hosts at UC Riverside Agricultural Operations. A sub-sample of 10 females per month was dissected to determine ovary rank of the specimens collected. Dissections of these female specimens reveal repeated patterns related to the proportion of previtellogenic females in the field. These patterns indicate two distinct generations each year with a possible third generation late in the season. Sampling will conclude in December 2004, and analysis will be completed to develop a model of female vitellogenesis cycles. A host plant study, completed in the summer of 2002, in which adult male and female GWSS were caged on grape, citrus, and oleander, has suggested differences in female fecundity and offspring survival. This study is currently being repeated. SEM studies have been completed and found a large number of sensilla on the female ovipositor. Morphology of these sensilla suggests that they may have mechanosensory or chemosensory functions. Histological studies of the female reproductive organs at varying stages of vitellogenesis are currently being analyzed.

INTRODUCTION
The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), is a serious pest of many tree and vine crops (Turner and Pollard 1959, Nielson 1968). The GWSS is of primary concern to California growers because of its capacity to vector the bacterium, *Xylella fastidiosa*, which causes vascular disease in a number of crops, including grapes, citrus and almonds, as well as landscape plants including oleander and mulberries (Meadows 2001, Hopkins 1989, Purcell and Hopkins 1996). An adult GWSS need only acquire *X. fastidiosa* once while feeding on an infected plant to then become a vector of *X. fastidiosa* for the remainder of its life (Frazier 1965, Purcell 1979, and Severin 1949).

Little is known about the reproductive biology of the GWSS. It has been reported that GWSS has two generations per year in Southern California (Blua et al. 1999). Oviposition occurs in late winter to early spring, and again in mid-to-late summer. Adult females can live several months and lay their eggs side by side in groups of about 10, ranging from 1 to 27 (Turner and Pollard, 1959). The greenish, sausage-shaped eggs are inserted into the leaf epidermis of the host plants.

Our research is focused on the reproductive morphology and physiology of the GWSS. We are examining the seasonal differences in female GWSS reproduction between summer and overwintering populations by studying oögenesis cycles. This knowledge is important in determining how GWSS might choose plant hosts in the landscape, which host plants are particularly good for GWSS ovarian development and why they are good, and finally how control measures might best be implemented based upon season and stage of reproductive development. Better knowledge of reproductive biology might also lead to better decision support including improved choices and timing of chemical or non-chemical approaches to GWSS control.

OBJECTIVES
1. Collect and prepare GWSS specimens for studying the morphology and anatomy of females.
2. Study and describe the sensory structures located on the female ovipositor.
3. Characterize the reproductive cycle of female GWSS in Riverside, California.
4. Study the effects of location on female GWSS reproductive cycle.
5. Study the effect of host plant type on female GWSS fecundity.

RESULTS
Oögenesis study
Female and male GWSS have been collected from July 2001 to September 2004. Samples were taken on monthly or bimonthly intervals. Dissections of female specimens collected from citrus hosts at UC Riverside Agricultural Operations have revealed repeated patterns related to the proportion of previtellogenic females in the field (Figure 1). In 2004, oviposition activity began in January with peaks in oviposition activity occurring in April and July. The proportion of young

(previtellogenic) females peaked in June 2004. The proportion of postvitellogenic females was highest in January 2004, followed by peaks in May and September. The patterns in percentage of previtellogenic, vitellogenic, and postvitellogenic females are similar to those observed in 2002 and 2003. These data suggest that GWSS may have two distinct generations per year. Our observations also indicate that although vitellogenic activity decreases in December, there is not a clear reproductive diapause in the population of GWSS in Riverside, California. The majority of the female GWSS that overwinter are postvitellogenic, suggesting that they have matured and oviposited before entering a reproductive rest period.

Histological studies of female oögenesis are being analyzed to verify the data collected from dissections. Morphological observations of the ovarioles are near completion, and the observations reveal that the ovarioles of the ovaries are the telotrophic type with asynchronous ovarioles.

Effect of Location on Number of Generations Per Year
We initiated sampling of GWSS populations in Tulare and Ventura Counties (California), but were unable to complete this objective due to strong eradication efforts which eliminated populations from our sampling sites.

Host Plant Study
The preliminary data of our host plant study in the summer of 2002 suggested that there is a potential difference in the female fecundity when caged on different plant species. For this study, adult female and male GWSS were caged on citrus, grape, or oleander, and allowed to mate and oviposit on the plants. We were successful in promoting GWSS oviposition and in rearing GWSS from egg to adult stage on all three host plant types. This experiment is currently being repeated with the late summer, overwintering generation of GWSS in citrus. Although the analysis is not yet complete, it appears that female fecundity patterns are different than those observed in the spring (early-summer) generation of 2002.

Scanning Electron Microscopy Studies
SEM study of the ovipositor has been carried out since September 2003. The SEM sessions have revealed sensory structures associated with the first, second, and third valvulae of the ovipositor. Many sensory hairs are also found to be located on the pygofer of the female. TEM studies are necessary to determine the exact type of sensillae present on the ovipositor. The external morphology revealed by SEM micrographs suggests that these structures include various types of mechanoreceptors and chemoreceptors.

CONCLUSIONS
It is too early this season to make any conclusions about host influences on female fecundity, but our prior data have indicated that female fecundity is influenced by host plant type. The observations suggest that it is feasible to target controls towards reproductive hosts (e.g. citrus) of GWSS in order to attempt to control future populations of GWSS. Although it appears that female fecundity varies between host plants, the fecundity may also depend on the generation (e.g. winter, spring, or early summer) being studied. Thus, it is important to avoid limiting year-long GWSS eradication efforts to those populations present on a single host plant type (e.g. citrus). In another experiment, we have successfully reared GWSS on a single host for two successive generations, under greenhouse rearing conditions. These greenhouse data suggest that multiple hosts are not necessary for the survival of GWSS. Thus, GWSS may not need to move between hosts in order to develop and reproduce. However, the pattern may change when GWSS are under field conditions where nutrients may be seasonally limiting.

More research on female host selection for oviposition is needed. Now that we have located sensilla that may function as chemoreceptors, it appears likely that there is a chemical basis for GWSS host selection. These sensilla may only function at close range, thus this knowledge may not be useful for trap development. However, the finding of chemosensilla on the ovipositor could be useful for future development of artificial media for GWSS oviposition in colonies maintained for parasitoid rearing.

Our study of the oögenesis cycle is defining the timing and number of generations of GWSS in California. This knowledge, combined with an understanding of female host selection, fecundity and offspring sex ratio, will result in a detailed understanding of host plant influences on female development and reproductive success. As indicated by somewhat conflicting results, based on the generation being studied, it is clear that the GWSS has complex reproductive patterns, and may have seasonally changing host preferences. Thus, it is important to modify eradication efforts based on the generation being controlled.

We are also beginning to understand the way in which GWSS may sense the environment and may be able to manipulate this system for monitoring trap development.

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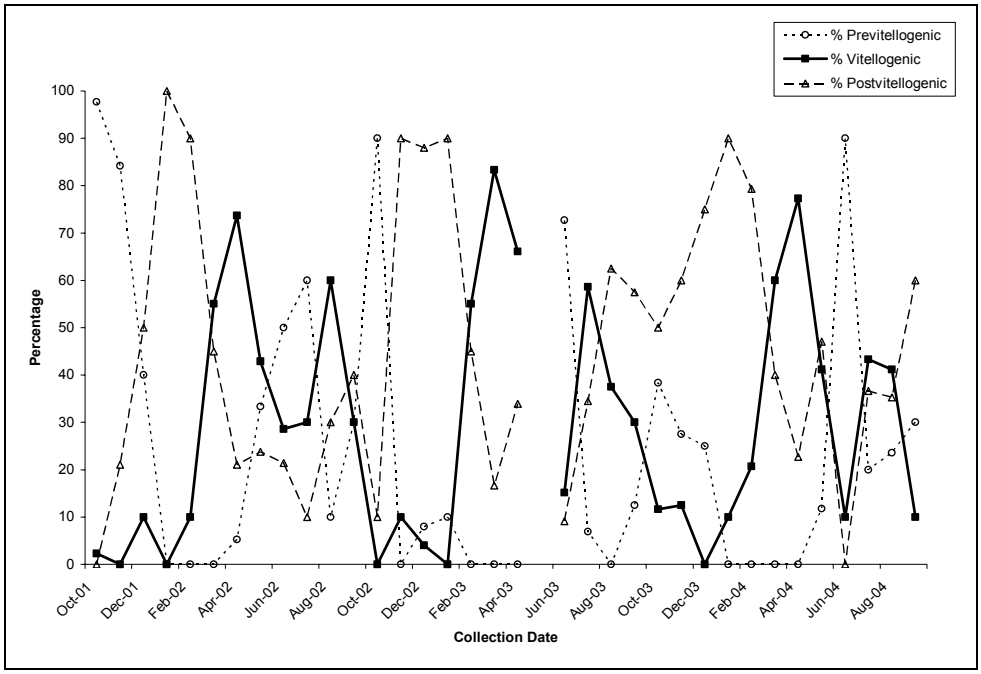

Figure 1: Percentage of previtellogenic, vitellogenic, and postvitellogenic adult female *H. coagulata* per month, according to dissections (October 2001 to September 2004), collected from citrus plants located at the University of California, Riverside, Agricultural Operations.

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GLASSY-WINGED SHARPSHOOTER IRIDOVIRUS PATHOGEN

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ABSTRACT
Pierce's disease of grapes, which is caused by the bacterial pathogen *Xylella fastidiosa*, threatens the national viticulture industry. The glassy-winged sharpshooter (GWSS) is the primary vector of Pierces disease which if not controlled threatens to completely eliminate the ability of the U.S. to compete in world markets. Viral pathogens of leafhoppers have yet to be examined as potential microbial control agents. Herein we examined the potential of a dsDNA virus, from the Iridoviridae, the iridescent insect infecting viruses, as a pathogenic agent of the GWSS. The GWSS adults were successfully infected with whitefly iridovirus, WFIV that had been propagated in *Trichoplusia ni* larvae. Virus infection caused reduced longevity and fecundity of GWSS. Adults were infected by microinjection and sprays. Infected individuals transmitted the virus to 'healthy' cohorts when caged together, suggesting an aerosol mode of transmission. Detection of virus positive eggs suggests that WFIV may also have a transovarial mode of transmission. Leafhopper vectors of Pierce's disease, such as the glassy-winged sharpshooter, *Homalodisca coagulata*, are susceptible to infection by iridescent insect viruses.